

Changing the objectives of research

The pressures on the British research enterprise, perhaps models of troubles that could arise elsewhere, are made worse by the government's lack of policy. But things may be changing.

WHAT follows is yet another account of the present agonies of the British system of publicly supported research, but that is not a signal to readers working elsewhere (by far the majority) to turn the page in search of something less parochial. For the British difficulty in adapting research and higher education to changing, and generally deteriorating, economic circumstances, is as vivid an illustration as there could be of the difficulties of changing course quickly in those fields. Even where the pace of economic growth is faster, so that the problems of containing spending within tight limits are less acute, there is plenty of evidence that the research enterprise is less flexible than it could be. Those who doubt that proposition should consider the condition of agricultural research in the United States, where attempts to reform the system going back for twenty years have mostly failed.

The British problem has its roots in the 1960s, the decade of relative prosperity when Mr Harold Macmillan, as prime minister, felt it proper to remind his electors that "you've never had it so good". That was the decade in which a threefold expansion of higher education was begun. It was also the decade when the British commitment to the European accelerator laboratory at Geneva was confirmed, and when the present pattern and scale of spending on civil science were determined more or less as they are at present. What else can have been expected of a decade in which the then Mr Harold Wilson (a later prime minister) offered before his election in 1964 a regenerated economy forged in the "white heat" of a technological revolution?

What has happened since is now a matter of record, much of it painful. Since the early 1970s, successive British governments of all kinds have been seeking to shed commitments on the grounds that they had become too expensive. The inquiry now under way under Sir John Kendrew to determine whether Britain should continue as a member of the European centre for high-energy physics (CERN) is not the first of its kind. Since the Rothschild reorganization in 1972, British government doctrine has been that the users of applied research should pay for it, with the result that many of the agents of research have become dependent for large parts of their budget not on the ministries that sponsor them but on a miscellaneous collection of other ministries with no administrative responsibility, either for efficiency or continuity. The present difficulties of the British Geological Survey, one of the largest identifiable components of the Natural Environmental Research Council, have arisen because its customers have become less willing spenders. Now the Agricultural and Food Research Council (AFRC) finds itself in the same case (see p. 8), facing the prospect that an as yet undetermined fraction of £10 million will melt away from its budget next year and probably twice as much in 1987-88. Similarly, British universities (and higher education generally) have for the past four years been made to survive on a budget unrelated to objectives of the enterprise, but instead determined as what the government considered it could afford.

Ignorance

Again in retrospect but also as a matter of immediate concern, the underlying difficulty is that British governments have set about the management of both higher education and civil science without sufficiently serious consideration of what these institu-

tion are intended to achieve. The present case of AFRC is a good illustration of how the government's attempt to manage this important part of the research enterprise is marked by what seems remarkably like ignorance of what research can do for an industry. It is not necessary to take sides in the dispute between the research council and the Cabinet Office about the yardsticks by which the scale of agricultural research should be determined to conclude that the system is being mismanaged.

The research council is the largest single agricultural research organization supported with public funds, and gets roughly half of its budget by way of research commissions from the Ministry of Agriculture, Fisheries and Food. But AFRC accounts for rather less than half of all the research supported by public funds in agriculture and related fields. Scotland, in this respect as in too many others, is separate. The ministry itself is responsible for fisheries research, forestry is parcelled out to the Forestry Commission while the ministry carries on its own books research laboratories intended to support veterinary services, among others. In short, there is a ragbag of arrangements for supporting agricultural research, which in normal times offers the advantages of plurality and diversity of research support, but when everything is being made to shrink makes it virtually certain that rationality will take second place after administrative convenience in decisions about which activities should be abandoned. That is one of the strongest reasons why the Scottish agricultural research stations should be united with those elsewhere in Britain, as a House of Commons committee recommended two years ago. The fact that the Scottish system is organized along the lines of the land-grant colleges of the United States (with researchers responsible for the provision of an extension service as well as innovations) should not be allowed to impede the more efficient distribution of effort and resources within this system.

Waste

The present system is also wasteful of such resources as there are. During the present financial year, AFRC will have spent more than £4 million on what is called "restructuring", the standard euphemism for the process of merging institutions and of persuading members of research staffs to take early retirement. Both in the research enterprise and in the British university system, this process has become a serious cause of wasted talents as well as money. As always, the people most willing to retire early are those who can most confidently expect to find something else to do, and who are likely by the same criteria to be most useful to the institutions from which they are displaced. While the theory of this process refers to something called the "managerial interest" by which applications for early retirement are judged, the academic world should by now be aware of the many talented British academic researchers who enjoy a premature pension and an academic salary from some other university system than the British. Is that a sensible way of hoping to turn a country's talent into national wealth? At the very least, the British government should be prepared to moderate the rate at which its research institutions are forced to contract by a sensible calculation of the speed with which that can be accomplished naturally.

Another irony running through recent experience of enforced contraction of the British system is that a government which is constantly complaining that academic research is indifferent to



the national economic interest is nevertheless apparently indifferent to the ways in which beneficial change might be accomplished. Thus the government's forecast of public spending published in January, which foreshadowed the further cuts which now threaten AFRC, boasts in passing that £2 million spent on the genetic improvement of pigs had yielded a benefit of £100 million a year to the pig industry, and that patented innovations in the design of agricultural mowers, themselves the products of a research programme costing £600,000, were yielding an annual income of £750,000 (to the British Treasury) by way of royalties. Sir Keith Joseph, who should know, was saying last week that the British government is soon to announce that such benefits will in future accrue to the organizations responsible — a promise first made in September 1983.

The issue is not merely one of equity, and of providing researchers with incentives to contribute to national wealth, but is also one of proper public administration. For how is it possible for people to make sensible decisions about the contraction of a research programme if they are required only to be concerned with costs? For AFRC, that issue is especially vivid in relation to plant breeding, for the council's excellent Plant Breeding Institute at Cambridge, which costs AFRC roughly £4 million a year, has recently been forced to cut back on staff ironically while the government is brooding on the question whether the National Seed Development Organization, which enjoys the right to market the institute's new varieties and which returned £2 million to the Treasury (half in tax) during its last financial year, should be turned into a private corporation. Why not lump the two institutions together, and let them make their own way in the world?

Core science

These lessons are not directly applicable to what remains the biggest upheaval under way in the British research enterprise, the attempt of the Science and Engineering Research Council (SERC) to remould its programme of research support so as to release funds for the support of what is called "core science" (physics research for example) while remaining within the limit of a budget shrinking slowly in real terms. Three months of agonizing have identified the options between which the council will have to choose at its meeting later this month. As elsewhere, no decision is likely to avoid diseconomies of one kind (wasting talent) or another (wasting money).

This research council exists exclusively for the support of academic research but has been progressively since the early 1970s caught in the trap that what seemed the sensible provision of facilities for academic research have become institutionalized, representing growing commitments within a budget either stagnant or declining in real value. Astronomy has been one of the council's successes during this period. From a virtually standing start, the British community finds itself with access to new instruments built with SERC participation in Australia, on Hawaii, and now at La Palma in the mid-Atlantic. In Britain, there are three substantial institutions dedicated to the support of these distant telescopes, the Royal Greenwich Observatory, the Royal Observatory, Edinburgh and the Rutherford Appleton Laboratory (which has its finger in many other pies as well). Not all of these can survive the month ahead. The betting seems to be that Britain will pull out of its Australian connection, leaving behind not merely the Anglo-Australian telescope but the excellent Schmidt telescope on the same site, operated for the time being by Britain alone. The obvious difficulty is that funds must somehow be saved, but that it would be too galling, and also uneconomic, to think of pulling away from La Palma, where construction is still under way. But even in the present complicated state of the politics of international observatories and of quotas of observing time, is it entirely beyond the bounds of possibility that a broader international base for construction of the La Palma observatory would keep more facilities alive and accessible? Back in the United Kingdom, it is also clear that one, the other or perhaps both of the observatories must go, but again it is important that neither talent nor money should be wasted for the sake of administrative tidiness. Should not SERC be looking for some

way in which the good work these laboratories do (in the development of instruments and the retrieval of data) could be transferred intact to universities, which are not often enough (in Britain) trusted with the execution of projects?

Another of SERC's problems is the clutch of machines that have been built as central facilities for university groups, but which are unlikely to be fully used in the years ahead for lack of funds for the general support of university researchers. There are lasers and synchrotron radiation sources, a neutron spallation source and nuclear structure facility. Again in retrospect, these machines were plainly commissioned too light-heartedly, without a sufficient calculation of whether SERC could afford to support enough customers to keep them fully used. The simple solution is that one or even two of these machines should be abandoned. The more complicated but better solution is that SERC should be enabled to shake free the shackles of chauvinism required of those who spend public money, and make whatever deals may be possible elsewhere in Europe for the continued use of facilities whose virtues, while not necessarily unique, are far from negligible.

SERC's biggest problem in the months ahead is, however, the decision that must be made about the subscription to CERN. The Kendrew committee has not yet reported, and may not until April, but there are only two things it can say — Britain cannot afford to continue to belong to CERN while the cost robs basic science of funds, but equally cannot afford not to belong. So there will be a period of further introspection, the issue will fall to be resolved by the government, which may or may not provide the extra cash that would be needed to square Kendrew's circle. But that will be a shabby way in which to deal with a serious problem — and what is in effect a treaty obligation. Walking out of CERN would not merely confirm mainland suspicions that Britain is a reluctant European but would damage an important laboratory at a particularly interesting time in its development. What the government (and SERC in its own interests) might reasonably expect, however, is that the future development at CERN, beyond the machine now planned (called LEP, an electron-positron collider), should be undertaken on a different financial basis, perhaps involving only the enthusiasts and the very rich. That way, the scale of SERC's dilemma would be limited in time.

What chance is there that the British government, hard-faced as it has been in the past five years, will listen to this special pleading? On past form, not much. But there is now at least a chance that the tide may be on the turn. After setting its face against the natural growth of student numbers at British universities, the government is now about to announce a planned increase, sufficient to yield about 1,000 graduates a year in science and engineering. Similarly, it seems the government has been more flexible than expected over the extra cost of continued membership of the European Space Agency. In each case the budgets of other ministries have been raided so as to provide urgently needed funds for education and science, necessary for carrying through policies on which the government has decided. Is it possible that a government whose reputation is for inflexibility has begun to appreciate that policies and not book-keeping should dictate the pattern of its spending?

That must be the hope. The British government's case for containing public spending within strict limits is strong. The need will continue for years to come. There may also be something in the government's constant complaint that the British investment in research has not yet been as productive of profitable innovation as might have been expected. But during the past five years, the management of the system has taken the form of publishing once every year figures showing what costs will be allowed and then grumbling from the sidelines. As anybody could have predicted, and as many did, the result within the system has been diseconomy. Nobody will suggest that governments, or even civil servants, should take on themselves the direction of research. But if they spend upwards of £500 million a year on these activities, they cannot walk away from the need, first, to help define their expectations and, second, to ensure that the resources are available. □

British higher education

Modest increase planned in student numbers

THE British government plans to announce next week an increase in the numbers of students following science and engineering courses at universities. This decision is the outcome of government alarm during the past two years at the shortages of trained people free for recruitment to British industry. But to many academics, the development will seem like the light at the end of a four-year tunnel.

The intention is that between £40 and £50 million should be spent over the three years beginning in September on the provision of extra places in science and engineering. Some of the money will come from the budget of the University Grants Committee, but the novelty of the plan (due to be made final at a meeting on Wednesday, 6 March) is that other government departments will be contributing. The Department of Trade and Industry and the Department of Energy are among those that have agreed to contribute from

resources they would otherwise have spent on their own account.

In one respect, the new development is an outcome of the plan, widely canvassed a year ago, for organizing a "switch" within British higher education from the arts and humanities to science and engineering. That was frustrated by the unwillingness of departments other than the Department of Education and Science to pay up. One feature of what is now intended is that the increase of science places (smaller than first planned) will not be offset by an actual reduction of the places available in the arts and humanities.

The scheme about to be launched will apply only to the universities. Although, in early discussions, the possibility was canvassed that polytechnics might also be included, representations by employers to the Department of Trade and Industry seem to have convinced the government that university graduates are most needed.

One of the questions to be decided on 6 March is whether the Open University and Cranfield Institute of Technology, which are supported directly by the Department of Education and Science, should participate.

The intention is that formal applications should be invited from universities during April, and that funds should be available both for supporting courses and for modest building projects. The urgency of the timetable the government has set for itself is dictated by the need to get things started at the beginning of the next academic year.

Among those participating in the planning of this new departure are some who fear that students with school-leaving qualifications of the kind required by most university engineering courses may not be available in sufficient numbers in the years immediately ahead.

Among academics, the chief import of the new development will seem to be the evidence it provides of a new flexibility in the British government's management of resources. Some think that the partial defeat last year of Sir Keith Joseph's attempt at redistribution within his own budget may have persuaded ministers that the system had become inflexible. □

Space budget fixed

A FEW weeks ago, the UK Science and Engineering Research Council (SERC) was beside itself with worry over how to pay an increased mandatory subscription to the European Space Agency (ESA), to pay for ESA basic science. But now, in an important decision of principle by the British government, it appears that SERC is to be taken off the hook.

The trouble began in January when European space ministers agreed a 10-year programme for ESA. The programme included a three-per-cent annual increase (eight per cent in UK cash terms) for the next five years in the ESA science budget. The straitened SERC, which belongs to the budget of the Department of Education and Science (DES), could not pay. Yet the subscription was due if the Department of Trade and Industry (DTI) was to participate, as it wished to do, in ESA's plan to join the US space station. The usual Treasury procedures did not allow DES to claim the increase from DTI.

Deadlock seemed likely. But now, according to DES, "SERC's additional costs are likely to be shared among various departments" — a statement that marks a fundamental breaking of interdepartmental barriers.

Meanwhile, discussions over a possible British "space centre" appear to be faltering over the location and powers of the centre. Several sites are being considered. Another less auspicious possibility is that the "centre" would be no more than a mere coordinating committee. Robert Walgate

US supercomputers

Four research centres named

Washington

THE National Science Foundation (NSF) has finally put its money where its governing board's mouth has been and awarded a total of \$200 million to set up supercomputer centres. They will be at the University of California at San Diego; Cornell University, in Ithaca, New York; the University of Illinois, Champaign-Urbana; and Princeton, New Jersey, near the campus of Princeton University.

recommendation was received more warmly by Congress than by the administration's budget-writers. So far, NSF's official response has been to buy time on existing supercomputers, underwriting the equipment, such as terminals and communications networks, that academic researchers have needed in order to gain access to those machines.

Under the contracts announced last week, the states in which the four centres

Location	Machine	Director
San Diego	Cray XMP	Sidney Karin
Champaign-Urbana	Cray XMP	Larry Smarr
Ithaca	IBM 3084 QX/ FPS 164s and 264s	Kenneth Wilson
Princeton	CDC Cyber 205 (after 1986, ETA-10)	Steven Orszag

NSF is to provide each centre with between \$7 million and \$13 million a year for the next five years for the purchase and operation of the equipment. Users will not have to pay for computer time, which will be allocated both by NSF directly — in the case of requests for large blocks of time submitted as part of NSF grant applications — and by research committees at each centre. NSF officials expect a roughly 50-50 split between these two.

Two years ago, a working group of the National Science Board, NSF's governing body, called for a \$200 million investment in ten supercomputer centres on the grounds that "important science" was already being neglected in the United States for lack of supercomputer capacity. The

are to be located will contribute an average of about \$20 million each. The host institutions will contribute about \$10 million each, mainly in land and buildings, while computer manufacturers will supply an additional \$50-\$70 million, mainly by giving equipment or providing it at reduced prices.

According to NSF, the four host institutions, together with 28 other universities that will be affiliated with the San Diego and Princeton centres through two consortia, will be able to "influence policies and procedures" of the centres but will have to compete on an equal footing with all users for time. The centres are expected to begin operating in late 1985 or early 1986.

Stephen Budiansky

US-Australian defence

Hawke fights on second front

Canberra

THE Australian Labor government seems to have successfully contained the damage that might have been done by last month's visit to Washington by Mr R. J. (Bob) Hawke, the Prime Minister. The issue is Mr Hawke's widely reported reversal of an earlier decision to provide logistical support for two US MX missile tests to be held this year in the Tasman Sea, off Australia's east coast. The government had feared that internal faction fights would cause trouble. Stiffer tests of consensus are to come, however. Mr Hawke has come under continuing pressure from the United States to strengthen US-Australian bilateral defence agreements and to promote the growing isolation of neighbouring New Zealand, following that country's refusal of port facilities to a nuclear-capable US warship earlier this year. The three countries are bound together militarily by the ANZUS treaty. On 4 March, however, Mr Hawke announced that the next ANZUS council meeting scheduled for July had been indefinitely postponed. Strictly speaking, under the treaty the three countries are obliged only to "consult together" in time of threat. But in practice ANZUS has been the formal basis for the US security guarantee to Australia and New Zealand for thirty-three years. Cancellation of the council meeting signals its likely imminent demise.

Mr Hawke's difficulties began immediately before his departure from Australia on 2 February for meetings in the United States with President Reagan and Secretary of State George Schultz, when he was reported to have agreed, without consulting his full cabinet or caucus, to provide facilities for US monitoring of the MX splashdown area.

The agreement would have fulfilled an undertaking by former prime minister Mr Malcolm Fraser, and would have made it possible to test the accuracy of the four-stage missile over its full range of 14,000 km, rather than over the shorter Vandenberg-Kwajalein range. With ten 350-kiloton warheads, the accuracy of the MX over a distance of 7,000 km is approximately 90 metres using NAVSTAR guidance.

Under pressure from the Labor Party's left wing, Mr Hawke cabled from Brussels on 6 February (the day before he was due to meet President Reagan) his decision to withdraw from the tests. The switch was made to look even more humiliating by Mr Hawke's exhortation three weeks earlier to New Zealand Prime Minister David Lange to rescind his decision to deny the use of port facilities to the destroyer USS *Buchanan* if the ship were to be carrying nuclear weapons, an item of information routinely refused by the US Navy.

Despite the MX fiasco, Mr Hawke has

been let off much more lightly by Washington than has Mr Lange. There appear to be several reasons for this. One is a desire to counter the growing influence of the Australian government's left wing, another to send a message to wavering NATO allies emboldened by Mr Lange's



actions, and a third the importance the United States attaches to its Communications Command Control and Intelligence (C³I) bases on Australian soil.

Information about these installations is scarce and more likely to be uncovered in US publications or under the US Freedom of Information Act than through Australian

government channels, but there are thought to be more than two dozen of them.

The continued presence of these stations in Australia has become a contentious issue. Critics of the facilities at Pine Gap and Nurrungar (see below) in particular charge that whatever their value when they were commissioned, during the "deterrence" years, they are now (or could become) part of a first-strike "counterforce" system and hence nuclear targets. This strikes at the heart of Mr Hawke's embattled strategy for peace and disarmament, a major plank of government policy.

The New Zealand government is soon to become even more isolated militarily after Mr Hawke's announcement last week that New Zealand will in future be denied the usually generous US component of the intelligence "product" formerly shared among the United States, United Kingdom, Australia and New Zealand and Canada under the 1947 UKUSA secret treaty. Practical problems of intelligence separation have arisen for the Australian government because intelligence from the "joint facilities" combines information from shared sources as well as that gathered solely by Australia. This is compounded by the presence (on secondment) of New Zealand intelligence officers in several sensitive Australian installations. Mr Hawke must now deal with the looming policy problems of south-west Pacific regional defence, at the same time avoiding the impression that Australia is dumping a small idealistic neighbour at the behest of the United States. **Jeffrey Sellar**

US defence facilities in Australia

OFFICIALLY referred to as "joint US-Australian facilities, the most important US defence installations in Australia are, according to Dr Desmond Ball of the Australian National University, the following.

Naval Communications Station Harold E. Holt, North West Cape.

Operational since 1967, this station maintains reliable communications with the nuclear-powered ballistic missile submarines of the US fleet patrolling the Pacific Ocean. The VLF (Very Low Frequency) facility is the largest and most powerful in the US worldwide communications system. North West Cape also has an array of high-frequency transmitters used for US military operations in the Indian and Western Pacific Oceans, as well as a ground station for the US Defense Satellite Communications Systems (DSCS). **Joint Defense Space Research Facility, Alice Springs (known as Pine Gap).**

Operational since 1969, the facility is 19 km south-west of Alice Springs and consists of seven large radomes and a large computer complex. Pine Gap was originally established as part of Project Rhyolite, which involves a small number of very large

antenna-carrying signals-intelligence (SIGINT) satellites in geostationary orbit. The facility monitors a wide spectrum of Soviet and Chinese military communications and radar transmissions, allowing the mapping of the extensive Soviet early-warning and air defence networks, as well as telemetry data transmitted during Soviet ballistic missile tests. This is the principal means of monitoring Soviet missile developments, and hence Soviet compliance with the strategic arms agreements.

Joint Defense Space Communications Station, Woomera (known as Nurrungar).

This is within the Woomera restricted area, 480 km north-west of Adelaide, and with Buckley, Colorado, is one of the two ground stations for the US satellite early-warning system. Nurrungar provides a real-time link between the North American Air Defense Command (NORAD), Strategic Air Command (SAC), the national military command system and the satellite early-warning system. Data are derived from infrared, charged particle and radiation sensors aboard the geostationary satellites of Program 647 of the Defense Support Program (DSP), which detect missiles shortly after lift-off. **Jeffrey Sellar**

Polish universities

Government legislation unwanted

Warsaw

THE "public debate" over Poland's 1982 Higher Education Act is becoming, more and more, an open confrontation between the universities and the government. A referendum last week among academic staff at Warsaw University gave an overwhelming vote of "no" to the proposed changes. (More than 75 per cent were against the reduction of the size of the senate, and more than 80 per cent against all other reforms.) A similar referendum among the students, now in progress, seems likely to give even more conclusive results. Yet the votes are unlikely to make any real difference. The government, it is believed, will simply push through the changes.

According to those who oppose the reforms, the changes would effectively annul the academic autonomy gained in the Solidarity period. Rectors and deans, at present elected, would be nominated by the Minister of Science and Higher Education, who would also have wide powers to suspend or dismiss staff, students and academic bodies. Political as well as professorial considerations would be taken into account in hiring university staff, and the "students' self-government committees" would be replaced by new bodies, based on the party-linked youth organizations.

The government case is that, since the state supports the universities, the state has a right to decide what goes on in them, and, in particular, to ensure that they are not used for anti-socialist purposes. The proposed changes in the act would therefore stress the explicitly "socialist" nature of Polish education, and wide disciplinary powers in the case of alleged "anti-socialist action"

by staff or students. (The definition of such action will, it appears, be wide according to sources in Warsaw Polytechnic. The rector of that institution is under pressure from the ministry to discipline students who carried the polytechnic's banner in the funeral procession of the murdered pro-Solidarity priest Father Jerzy Popieluszko, last autumn.) All that is happening, one ministry spokesman tried to explain, is that the Committee for Social and Political Affairs if trying to plug a few loopholes in the 1982 act, which have been used by opponents to destabilize the universities.

The academics, on the other hand, maintain that nothing is more likely to destabilize the universities than constantly changing the law.

The Main Council of Higher Education (which consists of one delegate from every university and higher college in Poland, with the exception of the Catholic University of Lublin) has repeatedly made the point that the present act should be allowed to run for the term of office of the rectors elected in 1984 (until 1987), before any changes are discussed.

One scholar formerly active in the unofficial Society of Academic Courses, the "Flying University" of the late 1970s, went so far as to say that what is at stake is not the election versus appointment of rectors, nor the existence of the "student self-government committees", nor even the right of the minister to suspend or dismiss recalcitrant staff or students. "What we are fighting for", he said, "is to ensure that the government cannot simply change the law every time it wants to interfere in the universities".

Vera Rich

UK research

Joseph warns of trouble ahead

THE whole of the British research establishment spent two hours at the Savoy Hotel last week to hear Sir Keith Joseph, the Secretary of State for Education and Science, deliver a speech whose preparation cannot have taken nearly as much time as that needed to feed the multitude. He was speaking at the annual luncheon of the Parliamentary and Scientific Committee.

Sir Keith had three points to make, of which the clearest was that the scientific community should address itself to the "moral dilemmas" occasioned by new developments in research. He said that the parliamentary bill introduced by Mr Enoch Powell MP, which would ban the use of human embryos for any purpose other than the treatment of infertility, is only "an introduction" to the troubles that lie ahead.

On support for research by the government, Sir Keith said that his partially successful attempt to transfer funds from student maintenance grants to research sup-

port had broken new ground by uniting all sections of his party in opposition. He went on to chide scientists in his audience because, he said, among the "uncounted" letters of protest about student grants, there had so far been found none — "not one" — applauding his attempt to increase research support.

Borrowing from C.P. Snow, Sir Keith also wrung his hands last week over what he called "the two cultures" of "science and trade". He complained of the "suicidal snobbery of academic attitudes" towards trade, arguing that the stagnation of British support for research is a consequence of poor economic growth in recent years.

But Sir Keith said there are now "harbingers of improvement" to be found both in the rate at which new enterprises are being created in Britain and in developments such as that of the complex of science-based industries recently grown up around Cambridge.

John Maddox

British AIDS

New cases cause alarm

SINCE the summer of 1982, 132 cases of acquired immune deficiency syndrome (AIDS) have been diagnosed in Britain, 58 of which have proved fatal. But not until last month was public anxiety about the disease fanned, following the publicity given to the death of a prison chaplain.

The Department of Health has now set up an expert advisory group representing science and medicine and public health services. The disease has not been made notifiable, although patients with AIDS are reported to the Communicable Disease Surveillance Centre by their doctors. Government policy is fivefold:

- Guidelines for all health professionals.
- Health Education Council leaflets to promote public awareness of the disease particularly among "at risk" groups (male homosexuals and intravenous drug abusers).
- Safeguarding of recipients of blood and blood products. Blood transfusion centres are distributing widely a leaflet *AIDS: Important New Advice for Blood Donors*; the British AIDS charity (the Terence Higgins Trust) and the Blood Transfusion Service are asking "at risk" people not to give blood.
- Development of tests to screen blood donations for the AIDS virus antibody.
- Speeding-up of the programme to produce heat-treated factor VIII (the blood-clotting factor) used to treat haemophiliacs. The Haemophilia Society is selling a comprehensive booklet about AIDS.

The government's financial commitment to AIDS research consists of £158,000 from the Medical Research Council (MRC) over three years (from October 1984), supplemented by £15,000 a year from the health department. MRC has also committed £27,000 for two years (with £7,000 a year extra from the health department) for research on the relationship of AIDS to patients with blood disorders. The US AIDS research budget is \$86 million a year (see *Nature* 28 February, p.725).

The AIDS virus has been isolated independently in three different laboratories and has been variously named the lymphadenopathy-associated virus (LAV), human T-cell leukaemia/lymphoma virus (HTLV) type III or AIDS-associated retrovirus (ARV). It is now recognized that all three are variants of the same virus (see *Nature* 313, 636 and 743; 1985) whose nucleotide sequences differ by 1 per cent and whose genetic organization is identical. The AIDS virus is thought not to be a member of the HTLV family of retroviruses, but to represent a new class.

According to Department of Health figures, 90 per cent of deaths due to AIDS in Britain have been male homosexuals of average age 36.

Maxine Clarke

Genetic manipulation

Rifkin battle lost, war undecided

Washington

A PANEL of the US Court of Appeals has ruled invalid a lower-court injunction forbidding the National Institutes of Health (NIH) to approve any deliberate-release experiments (see *Nature* 312, 689; 1984). But the Lindow experiment on frost-resisting bacteria is still held up pending approval of a formal document assessing its environmental risks.

The lower-court injunction, granted to anti-genetic-engineering activist Jeremy Rifkin, had ordered NIH's Recombinant DNA Advisory Committee (RAC) to prepare a lengthy "environmental impact statement" before considering any other proposals for field trials of recombinant organisms. NIH officials said that preparing such a document would have taken a year and would itself have been subject to further legal challenges.

The Lindow experiment, in which genetically-modified bacteria would be sprayed on potato plants to protect against frost damage, was to be the first in which bacteria containing recombinant DNA were to be released to the open environment. The experiment was approved by RAC in June 1983; in August of that year, Rifkin sued in federal court to halt it.

Although the appeals court has removed the biggest threat that Rifkin held over RAC — the prospect of tying up all deliberate release experiments in years of legal manoeuvring — it left no doubt that NIH did not follow the law in approving the Lindow experiment without first completing a formal environmental assessment. The court said that environmental assessments would likewise be required for each deliberate-release experiment that comes before RAC.

As for the Lindow experiment, it is now apparently up to the lower court in the person of District Court Judge John Sirica to decide whether the environmental assessment that the RAC staff has in fact just completed on the experiment is adequate. Rifkin's lawyer has raised the possibility of challenging that point. Rifkin himself has said on several occasions that he does not believe that there is at present sufficient knowledge fully to assess the environmental risks posed by the deliberate release of recombinant organisms.

The court's ruling also opens the way for routine legal challenges of every deliberate-release experiment that comes before RAC on the grounds that the environmental assessment is inadequate.

NIH are meanwhile taking steps to get out of the business altogether. The Environmental Protection Agency (EPA) intends to regulate all field trials of "microbial pesticides" that have been genetically altered or manipulated; under new EPA rules, the agency would have to be given 90 days' notice of any such test.

(Previously, EPA regulations applied to field trials of pesticides only when the test plot exceeded 10 acres.)

EPA is also working on regulations that would apply to field trials of other recombinant organisms as well. At the next RAC meeting, scheduled for 3 May, the committee is expected to approve a proposal that would allow researchers subject to the RAC guidelines (currently any institution that receives federal funds for recombinant DNA activities) to go ahead with deliberate release experiments so long as they have approval from another federal agency; RAC approval would no longer always be required.

RAC has not received any new proposals for deliberate-release experiments from

federally-funded institutions since the Lindow approval went to the courts. Two private companies, however, Advanced Genetic Sciences (which proposed an experiment virtually identical to Lindow's) and Cetus Madison (which proposed a plant experiment), voluntarily submitted proposals last year; RAC approved them, but the NIH director, James Wyngaarden, decided that even though the injunction did not apply to private companies (which are not legally bound by the RAC guidelines), there should be no double standards, so he is holding up approval until environmental assessments are prepared for them. Advanced Genetic Sciences has since withdrawn its proposals and has decided to deal only with EPA; Cetus Madison has been told that if it still wants its proposal considered by NIH, it should submit a draft environmental assessment.

Stephen Budiansky

Court wishes plague on both sides

THE following are extracts from the opinion of the United States Court of Appeals in the case in which NIH appealed from a ruling of the lower court awarding an injunction on deliberate-release experiments to the Foundation on Economic Trends, Mr Jeremy Rifkin's organization.

Judge Wright

This appeal presents an important question at the dawn of the genetic engineering age: what is the appropriate level of environmental review required of the National Institutes of Health (NIH) before it approves the deliberate release of genetically engineered, recombinant-DNA-containing organisms into the open environment?

We emphatically agree with the district court's conclusion that NIH has not yet displayed the rigorous attention to environmental concerns demanded by law. We therefore affirm the district court's injunction prohibiting the University of California deliberate-release experiment until an appropriate environmental assessment is completed. We also share the district court's view that NIH should give greater consideration to the broad environmental issues attendant on all deliberate release of organisms containing recombinant DNA, and to its own responsibility for approving these deliberate-release experiments. We find, however, that the part of the injunction enjoining NIH from approving all other deliberate-release experiments is, at this juncture, overly broad, and we therefore vacate the part of the injunction that prohibits NIH approval of those experiments. We emphasize, however, that if NIH fails to give appropriate environmental consideration to any other experiment, as it has failed to do with the University of California experiment, injunctive relief would be clearly proper.

Broad claims are made about both the potential benefits and the potential hazards of genetically engineered organisms. Use of

recombinant DNA may lead to welcome advances in such areas as food production and disease control. At the same time, however, the environmental consequences of dispersion of genetically engineered organisms are far from clear.

The Lindow experiment

On September 17, 1982, Drs Lindow and Panopoulos, scientists at Berkeley, submitted a request for NIH approval of an experiment that would involve deliberate release of genetically altered organisms in the open environment. NIH approval was required because the University of California receives NIH funds for recombinant DNA research.

Neither the government nor the university seriously disputes that an environmental assessment is necessary. The university's contention here is that the environmental consideration given by NIH was equivalent to the necessary environmental assessment that the injunction requires only a document labelled "Environmental Assessment".

We thus fear that the university and the government completely misapprehend the district court's holding and the requirements imposed by NEPA [the National Environmental Policy Act]. The most glaring deficiency in NIH's review of the Lindow-Panopoulos experiment is its treatment of the possibility of dispersion of recombinant-DNA-containing organisms. An environmental assessment that fails to address a significant environmental concern can hardly be deemed adequate for a reasoned determination that an EIS [environmental impact statement] is not appropriate.

NIH procedures

The district court also found that plaintiffs were likely to succeed in showing that NIH should have completed an EIS (1) in 1978, when it revised the guidelines to make deli-

berate release possible, and (2) in 1982 or 1983, when it became clear that NIH would be receiving proposals for deliberate release. The district court thus enjoined NIH from approving all other deliberate release experiments. The emphasis on the 1978 revision is misguided for two reasons. First, the policy revision did not irrevocably commit NIH to any decision. Second, significant changes have occurred since 1978, including the maturation of genetic engineering to a point where deliberate release is feasible and imminent. It makes little sense to rest a judgment on *prospective* relief on decisions made several years ago in different circumstances.

The Supreme Court has emphasized that the question of preparing a programmatic EIS [which plaintiffs argued NIH should have prepared in 1982 or 1983] is initially committed to the agency. We are not prepared to agree that plaintiffs are likely to succeed in showing that the absence of a programmatic EIS unreasonably constricts adequate environmental evaluation.

Judge MacKinnon, concurring

I am of the opinion that the foundation should have made its original application to NIH. This is not a case of just failure to exhaust [administrative remedies], but a case of a complete failure of the foundation to present any claim whatsoever to the agency — NIH.

I can understand how the RAC scientists who are knowledgeable in this field of genetic engineering would approve the experiment by a vote of 19-0 with no abstentions. It would seem that an experiment that releases into the environment organisms substantially the same as some already living there, and subject to the same naturally occurring controls, would present no risk. However, the general public and those who have to pass on this action are not knowledgeable in this field and they are easily frightened by new scientific experiments and their possible consequences. It is such lay concerns that must here be satisfied by Environmental Assessments and Impact Statements.

Due notice of the pendency of this matter was given in the *Federal Register* and comment was invited, but none was forthcoming. Had the objections of the foundation been alertly raised before the agency, the district court and this court would undoubtedly have had a better record to consider and might even have been spared the necessity of ruling on the case.

The foundation's conduct has also delayed this vital experiment for a very considerable period of time. The use of delaying tactics by those who fear and oppose scientific progress is nothing new. It would, however, be a national catastrophe if the development of this promising new science of genetic engineering were crippled by the unconscionable delays that have been brought by litigants using the National Environmental Policy Act and other environmental legislation in other areas. □

Star wars

Quarrel over congressional study

Washington

THE Congress's Office of Technology Assessment (OTA) has reacted angrily to accusations that its study of President Reagan's "star wars" Strategic Defense Initiative (SDI) is "designed to kill SDI rather than examine it". The accusations were made last week by Lieutenant General Daniel Graham, a former member of the advisory panel overseeing OTA's study and a prominent champion of space-based missile defences. OTA says there is "no basis whatever" for Graham's attacks and accuses him of breaking faith with OTA's study by distributing confidential documents to senators and to a senior official in an (unnamed) executive agency.

Graham is director of the High Frontier organization, which is widely thought to have been influential in persuading President Reagan to instigate research into missile defences. Graham resigned from the OTA panel on 2 February after being told

by OTA's director John Gibbons that he must undertake to keep OTA study documents confidential in order to remain on the panel. Graham admits distributing confidential OTA documents annotated by himself to three senators and the executive official. Graham's explanation for his action is that "this concerns national security and I consider that more important than their damn procedures".

Graham's charges extend both to OTA staff, whose study drafts he describes as "utterly biased", and the advisory panel, which he says is "heavily stacked with highly vocal opponents of SDI". OTA replies that Graham chose to list only those members with whom he personally disagrees; the panel has 21 members in all, chosen to represent all major points of view. Sidney Drell of Stanford University, a critic of SDI who appears on Graham's list, dismisses Graham's charges as "patently false". Another accused panel member, Richard Garwin, praised the work of the OTA staff and the panel chairman. The panel's chairman, Guyford Stever of Universities Research Associates, did not wish to comment on Graham's charges in the interests of maintaining neutrality.

One section of OTA's draft report which particularly incensed Graham lists possible goals of SDI and evaluates them. According to Graham, OTA "stated that a goal is to provide means for the United States to credibly threaten the use of offensive nuclear weapons should it so choose". OTA replies that Graham's quote is taken out of context; the draft did not suggest that this was the actual objective of SDI. Another of Graham's objections is that the report "attempts to make the issue a choice between the Anti-Ballistic Missile (ABM) treaty and SDI".

The issue that brought to a head the row between Graham and OTA was an attempt in the report to separate the issues of anti-satellite weaponry and ballistic missile defence. Graham says this was done to weaken the case for SDI; after unsuccessfully challenging OTA's authority to do so, he took his arguments to senators, and at least one senator has made representations to OTA about the study, says Graham.

OTA is no stranger to star wars controversy; last year, a background paper prepared for the study by Ashton Carter of Harvard University was vilified by Lieutenant General James Abrahamson, director of SDI. Carter's report concluded that the prospect of a near-perfect defence against nuclear missiles was so remote that it should not form the basis of public policy. Graham says he hopes his resignation will serve to discredit the latest OTA report; on the other hand, as one panel member pointed out, it also means there is now one fewer SDI supporter on the panel.

Tim Beardsley

British cooperation

Washington

FOLLOWING the recent endorsement of SDI research by British Prime Minister Margaret Thatcher during her visit to Washington, negotiations are now under way here to determine how Britain might best cash in on the effort. The SDI office has made it plain that it welcomes allied research cooperation ("we need all the help we can get") and says "the ball's in their court". One major obstacle, however, is that Article IX of the ABM treaty, signed by the United States and the Soviet Union in 1972, expressly forbids the transfer to third parties of anti-ballistic missile technology covered by the treaty. A statement agreed between the signatories makes it clear that the article covers the transfer of "technical descriptions or blueprints specially worked out for the construction of ABM systems or their components". But Britain, and presumably other allied nations, would seek agreements guaranteeing just such transfers.

The British negotiations in Washington are being led by Dr John Green, deputy head of the embassy's defence staff. One option is that a memorandum of understanding might be exchanged between the two governments; an objective of the negotiations is to establish a coherent framework for British research institutions and companies wanting to offer their expertise to the SDI office. As cost does not seem to be a major object in SDI, there should be plenty of opportunity for qualified British scientists to jump on the bandwagon. But first there will have to be a detailed scrutiny of research capabilities in relation to needs; the results are likely to remain classified.

Tim Beardsley

Agricultural research

British council under new threat

THE least favoured of the British research councils, the Agricultural and Food Research Council (AFRC), is faced with yet another period of contraction. At the end of a year in which it has shed 550 jobs, one-sixth of the total, the council is now faced with the prospect of a further reduction of research commissioned by the three agriculture ministries, the Ministry of Agriculture, Fisheries and Food and the ministries for Scotland and Northern Ireland.

This is the gloomy theme of the second of the council's corporate plans, published earlier this week. Sir Ralph Riley, the secretary of the council who retires on 1 April, says in his preface to this document, which will become the basis of the council's claim on public funds in the financial year beginning in April 1986, that a revised plan may be necessary when the government's intentions are known.

The discontent of successive British governments with the council's work goes back at least to an internal inquiry in the late 1960s. At the time of the Rothschild reorganization in 1972-73, AFRC was the most directly affected, being required to depend on commissioned research from the

agriculture departments for 75 per cent of its budget. In the past decade, the volume of commissioned work has steadily declined, as has the council's share of the science budget (from which all research councils are supported). Two years ago, the council formally dissented from the annual allocation recommended by the Advisory Board for the Research Councils (ABRC).

In the financial year now ending, AFRC and the Scottish institutes will have received £46.7 million from the science budget and the remainder of the total of £121.6 million by way of commissions. The planned increase of the cash budget for 1984-85 to £125.6 million (not fully compensating for inflation) includes an increase of £3.6 million from the science budget and of £0.5 million in commissioned research.

Government forecasts of public spending published last December show the total budget standing at £126.8 million three years from now (in 1987-88), but the corporate plan says that even this static budget has now been put in doubt by the planned reductions of departmental research and development of £10 million in 1986-87 and of £20 million in 1987-88.

The council is particularly sore at the way in which a Cabinet Office paper published at the end of last year used a comparison of agricultural research costs and output values in the countries of the Organisation for Economic Cooperation and Development (OECD) to demonstrate that Britain spends a greater proportion of the value of agricultural output on research and development than other countries. The assertion was quickly denied (*Nature* 3 January, p.8), and the corporate plan published this week says that other indicators would lead to different comparisons. Yet senior officials of the council fear that the Cabinet Office comparison is still being "hawked around Whitehall" to try to discredit its programme of research.

For the rest, AFRC seems not to have won the moral support it might have expected from the newly-formed Priorities Board on Research and Development in Agriculture and Food, which has apparently been asked to consider what steps could be taken to increase industrial sponsorship for the council's research. The board has apparently taken the line that the central government should pay only for what others cannot be expected to pay for.

The corporate plan says that if the council's budget is further reduced, "further restructuring" may be necessary. During the past few years, this has chiefly meant the closing of research stations and the shedding of staff, but now there is also talk of allowing some parts of the agricultural research enterprise to become free-standing. The possibility that there may be a merger of AFRC and the Scottish research stations is also being explored. □

Planned contraction

THE chief ingredients of the new corporate plan for agricultural research, covering both the work of AFRC and the Scottish stations supported directly by the Department of Agriculture and Fisheries for Scotland are as follows:

University research. The plan is that spending on research grants (increased by 8.5 per cent to £5.4 million this financial year) should continue to take a larger share of total funds. By 1989-90, the plan says, £7 million a year will be spent in this way, with priority given to food research.

Food science. The plan says that AFRC has warmly endorsed the recommendations of the Advisory Council for Applied Research and Development (ACARD) in 1983 that more attention should be paid to food science. Research spending will increase from £13.3 million this year to £19.3 million five years from now, or by 26 per cent in real terms. The plan is to concentrate this work on the three research stations at Bristol, Norwich and Reading, but other council research institutes will also be involved.

Animals research. The intention is that spending on animal diseases should decrease by 16 per cent (to a cash value of £16.4 million a year) over the next five years. Part of the council's recent restructuring has entailed the merging of the Grasslands Research Institute and the National Institute for Research in Dairying at a new site at Hurley, in Berkshire. During the coming year, the Animals Research Institute at Cambridge will be merged with its parent station at nearby Babraham.

Forage crops. The corporate plan outlines a strategy on concentration of research under the umbrella of the Animals and Grasslands Research Institute, with the Welsh Plant Breeding Research Institute and its two Scottish equivalents taking more tightly defined roles within the whole. Plant protection research will decline in the next five years by 16 per cent.

Capital development. The plan says that the recent tendency to spend 12 per cent of the AFRC budget on buildings "cannot be sustained", and that it will in future not be possible to replace some obsolete equipment or even to provide the material support for some new research programmes. But the council plans to earmark £3.5 million from the budget for the year after next to support initiatives not yet proposed.

Manpower. In the year now ending, 100 of the 550 posts lost from the AFRC establishment have been compulsorily vacated. The earlier contraction of AFRC, not yet completed, entails the loss of a further 250 posts during the coming year; the new plan says that it will also be necessary to lose a further 800 posts in the next two years, offset by an increase of 60 posts for food research. The rule that people should retire at age 60 will be strictly enforced, and as many as possible of those not needed will be persuaded to take early retirement. □

Schopper to stay

THE European Organization for Nuclear Research (CERN), near Geneva is to be directed for a further four years by the present incumbent, Professor Herwig Schopper of West Germany. He will thus remain director-general until the end of 1988, which should see the end of the large electron-positron collider (LEP) project.

Italy, which has been campaigning for a strong place in the directorate, has won a place for Professor Carlo Rubbia, Nobellist for his discovery of W and Z particles with his CERN proton-antiproton collider. Rubbia will chair a working group which will study "the long-range scientific and technological future" of CERN.

Meanwhile, after a meeting on the latest proton-antiproton collider results in St Vincent, in northern Italy, last week, physicists were surprised by the continuing discovery of events producing pairs of muons each carrying the same electric charge, but moving in opposite directions. A total of seven such events have now been seen in which the muons are not associated with "jets", the now-familiar signal for quark production and decay. Four of the events have no jets at all, and in five of the seven, strange quarks are produced (double the expected rate). No satisfactory explanation for these "like-sign dimuons" has been offered. Rubbia described these results and others as "a lot of good things cooking". However, "we must be prudent" in their interpretation.

Robert Walgate

European research

Commission's new broom

Brussels

KARL-Heinz Narjes, the ex-U-boat officer who is now vice-president of the European Commission and responsible for industry, information technology and science, believes that the Commission's research policy is under test.

"If we fail this test, we will have a major political setback" said Narjes last week, sitting at pentagonal table at which commissioners representing the ten nations of the European Community regularly attempt to plan Europe's future.

A lawyer, and one of the founding fathers of the European Community, Narjes also firmly backs Esprit, the ambitious European programme of research in information technology started by his predecessor, Belgian Viscount Etienne Davignon. At the immense cost (by Commission research standards) of some £80 million a year, exceeding Europe's nuclear fusion research effort for example, Esprit attempts to knock European computer and electronics companies into some kind of coherent research policy and common standards.

Some major companies, such as Olivetti of Italy, have effectively spurned Esprit and believe they can dominate Europe with the help of US allies such as AT&T, while others continue to believe the programme can help (by forcing communication and joint standards). And for Narjes, "certainly Esprit is one of the tests". Moreover, "it is going very well", he claims. "Five hundred researchers are involved this year and there should be 2,000 next, and the first patent has already been applied for."

The setback in December when the European Parliament rejected the Commission's budget for 1985 will not much affect Esprit, the Commission claims, because the bulk of last year's money can be carried forward to 1985. On the other side of this coin, however, it seems the programme has been very slow to get moving.

Nevertheless, the Commission's political masters, the European Council of Ministers, had given a vote of confidence in Commission research programmes on 19 December with agreement in principle to support a substantial expansion of eight major programmes. Biotechnology, for example, will grow from its current budget of £6 million to £30 million a year. The Commission asked council for £2,000 million for 1984-87 and received a promised £1,400 million, still a substantial increase on current spending. "There's relief at being on the move again", said deputy research director-general David Hywell Davies last week. The test, said Narjes, is "whether we can manage the sums allocated".

But Narjes is already looking beyond this "first phase of solidarity" of European

research towards the "horizontal problems". These are the mobility of scientists and "the need to expose them to challenges".

Narjes sees no easy solutions. There are mundane obstacles such as national insurance, pensions, work for a spouse, differing qualification and education systems, security. Language itself is an "underestimated" problem, says Narjes. And it is politically difficult to argue that scientists should be treated as a special case, although the current political interest in using science to solve the economic crisis has given scientists an opportunity that they should seize. "We must make a major, long-term effort" for mobility, says Narjes. Does he have any real hope of success? "We have experience of hard negotiations. We can be 10 per cent tougher than the toughest member state."

Narjes also emphasized:

- The poor access of some non-English-speaking scientists to major US research journals.

- BRITE, a Commission programme to inject new technologies and science into small and medium-scale industries, which will be "a key programme if it succeeds".

- Agriculture, which takes 70 per cent of the Commission's budget "despite serious reforms", where the new biotechnology programme may help to give farmers new economic non-food products.

- The "budget needle", the eye of the needle through which Commission research projects must pass (the total budget still lies around 2-3 per cent of national research programmes). Here "we are seeking other sources" in the Commission, Narjes hinted.

- Big European research machines, such as the politically troubled European Synchrotron Radiation Source, for which the Commission might consider buying a "users' ticket" to distribute through Commission programmes.

- Coordination of Commission research programmes such as BRITE with space station research.

- Intra-Community communications, in science as in other fields, which form "one of the major European problems".

Robert Walgate

European cooperation in the air

BRITE, the European Commission's programme of Basic Research in Industrial Technologies for Europe, may have been oversold by its enthusiastic directors — but it has stolen a march on some other Commission programmes.

According to Dutchman Hendrick Tent, programme head, there has been "overwhelming reaction" to BRITE's invitation to industry and research bodies to participate in projects that will improve production techniques and provide the research base for new product lines in the next 5-10 years.

BRITE has been promised £70 million over four years, to be doubled by industrial contributions, but, like all the new Community research programmes, it still awaits final official approval from the council of ministers of the member states of the Community. This is expected any day now. But BRITE did not delay, having already called for "expressions of interest" to speed up the often bureaucratic process of calling for tenders and choosing and defining final projects. The BRITE team already has a good idea of what projects will be submitted, and has helped companies and researchers to prepare appropriate ideas and consortia, which contain partners from two or more member states and at least one industry. The programme has so far identified a need for projects in reliability and wear, laser technology, joining techniques, testing procedures, computer-aided design, electrochemistry, catalysis and particle technology.

Of the 3,037 expressions of interest received by 31 December, more than half

were from industry, and more than a quarter from applied research organizations such as TNO from the Netherlands and the German Fraunhofer Gesellschaft. Fewer than a quarter came from academic institutions, although the proportion was higher in Britain. Britain made the most replies: around 800, compared with 700 from Germany, 500 from France, 400 from Italy, and 100 from Ireland. Some 21 applications came from the Community's newest member, Greece.

- The European Commission's "stimulation" programme aimed at getting European academic laboratories together rejected 90 per cent of the 700 applicants for its first experimental phase. And it may have to do the same in 1985, the first year of full operations even though its support should increase fivefold (according to the 19 December council of ministers decision).

So said one of the stimulation programme administrators in Brussels last week, explaining that the demand for European integration at academic level — and the concomitant funds — is enormous.

The stimulation programme should now get at least £33 million for 1985-89 (compared with just £4 million for 1983-84), of which £20 million is to be spent in the first two years, when ministers have encouraged the Commission to reapply for an increase. Sixty per cent of the cash will be spent on the twinning of laboratories, but some cash will also be put aside to run a European version of the "Gordon conference", where a select group of scientists can brainstorm in privacy.

Robert Walgate

NERC's corporate plan

SIR — The leading article (*Nature* 14 February, p. 515) on what you call the prospectus for the Natural Environment Research Council (NERC) contains a good breakdown of our present problems. Your conclusions do not follow. Having made the case for greater cohesion in the field of environmental research and touched on the history of why there is none, the answer is not as you suggest the dismemberment of NERC.

Early drafts of the corporate plan had the idea of submerging the research institutes in the new science divisions. Greater cohesion and flexibility may well come from abolishing the institutes, at the cost of more centralization. The plan published on 14 February was ambiguous. The absence from NERC of mainstream meteorological work is serious, but the corporate plan seeks to include work on climate. The main aim of the plan in its present form is to preserve university grants at a meaningful level. You rightly point out that in five years the universities will hardly notice the benefit. In contrast, in the same period, the funding to institute research could fall by up to 50 per cent and much long-term research will have been written off.

In the British Geological Survey (BGS) many staff feel a new home is necessary. They are committed to the need for continued survey but cannot carry out that task without an adequate level of dedicated funding. BGS finds no stability of finance within NERC and has been the target for an unfair share of cuts already. The proposal that BGS could find an umbrella similar to the British Antarctic Survey is as illogical as it is unlikely. No obvious slot exists for BGS and the present government would never sanction either the making of a new quango or the subsuming of 1,500 extra civil servants into an existing department.

The position of BGS outside central government gives a freedom with which only privatization might compare. The disadvantages of the latter course to the majority of staff and to the scientific needs of the community are obvious.

In the case of the Institute of Terrestrial Ecology (ITE), it is now untrue to say that any greater research links exist with the Nature Conservancy Council (NCC) than with any other government agency with interests in the biological environment. Scientists in ITE see a much wider role in the applied field of pollution studies and all aspects of land use, notably forestry and agriculture. The Ministry of Agriculture, Fisheries and Food and the Agricultural and Food Research Council have been slow to encompass sound ecological principles in answering environmental concerns. The House of Lords Select Committee report on agriculture and the environment (1984) came out in favour of much better liaison

in research. Any return of ITE to a functional link with NCC would restrict the research scope and increase the accusation that the institute is simply an advocate of nature conservation. This denies the relevance of ecological work to a whole range of human activities. In any case research should be conducted away from the shackles of departmental policies.

Returning to the leading article of 14 February, your analysis of events leading to the NERC corporate plan was right, but the basic problem is the government's insistence on cuts. As its prescription may not change, it is best to look at what can be salvaged.

I have stated above why for dogmatic reasons the carving up of NERC will not suit the government either. It may be possible to persuade it to accept the proper integration of environmental science in the United Kingdom. In doing so it may get economies and take out some duplicated effort. To allow the fragmentation of NERC would make even more tenuous the links between disciplines and weaken research effort in key areas. This must not happen. Though we are against the planned 30 per cent cut in NERC manpower, we support the need for a properly directed and capitalized research council in the United Kingdom. It is our belief that reduction of institute strength and further fragmentation of research is not the way forward.

RICHARD SCOTT
(Chairman, Union side)

*Institute of Terrestrial Ecology,
Merlewood Research Station,
Grange-over-Sands,
Cumbria LA11 6JU, UK*

SIR — Given the now unavoidable association between the Natural Environment Research Council (NERC) corporate plan and the notion that a 3,000-strong workforce is to be reduced to 2,000, the choice of St Valentine's Day to announce the contents of such a melancholy document seemed at first sight inapposite until it is remembered that this feast day for romantics also commemorates a notorious massacre.

It may be about to commemorate another one — the sacrifice of the institutes on the altar of the universities. One of the principles underlying the plan and one that appears to have become axiomatic and therefore apparently beyond discussion (see *Nature* 31 January, p.340), is the transferring of institute resources to make up for the shortfall in government financial support for the universities. The plan is obviously modelled on the Mason report into the research councils which made canonical the notion that university research was more flexible and therefore better than the research of the institutes.

Nowhere is the term "flexibility" defined or explained. It must be assumed in the absence of an explanation that flexibility in some way refers to an organization's response to the changing times we live in. In other words, it seems flexibility may mean how organizations respond to what has become almost the universal arbitrator — the market forces.

Terrestrial and fresh water ecology seem to have been earmarked for a large contribution towards the "Save the Universities Fund", yet the Institute of Terrestrial Ecology (ITE), for example, was born out of a not dissimilar transfer of funds a decade ago when a substantial percentage of its money was handed over from the Department of Education and Science with the Nature Conservancy Council (NCC) to the Department of the Environment, where NCC was to be a major customer of ITE and any other organization that chose to tender for NCC contracts. ITE has had to compete, and still does compete, with the universities among others for that money. If the universities are not able to obtain enough of that money through competition, are we to understand they will instead be given a proportion of, in this instance, ITE's funds instead? If this is so, how does this principle of flexibility operate other than to appear as a word whose context leaves it without meaning but with a use, namely that of a magic wand with which to shrink institutes.

In place of a more satisfactory explanation of the apparently fundamental concept of flexibility, perhaps there should be much wider consultation, not only on the "details" (*ibid*) but on the principles of the corporate plan.

Otherwise the more cynical employees of NERC institutes may just come to the conclusion that, since the institutes have no direct voice on council and the universities have many, what is being witnessed is not a "rational reconstruction" but something quite different.

BARRIE PEARSON

*13 Knoll Cottage Residential Park,
Winfrith, Dorchester,
Dorset DT2 8LD, UK*

Embryo research

SIR — The question "who can object to the proposition that there can be no such thing as a living being without implantation?" posed in your Opinion column (*Nature* 21 February, p.612) fails to address the objections to embryo research. In my mind the issue is not the point at which an embryo becomes a viable organism, but rather the point at which a unique potential member of our species is created. There can be no objections to the proposition that this takes place at fertilization.

ROGER WATSON

*14 Eldon Avenue,
Shirley,
Croydon CR0 8SD,
Surrey, UK*

An appeal to embryologists

The British House of Commons seems bent on passing a bill to prevent some kinds of research. Here is a way in which embryologists and geneticists can help themselves and even help defeat the bill.

MR Enoch Powell's already famous bill to prevent the use of fertilized human embryos for any other purpose than the treatment of infertility seems to have carried the British House of Commons with it. The substantial majority for the second reading of the bill two weeks ago, endorsement of the principle, is an embarrassment for the British government, properly anxious to produce a comprehensive piece of legislation to deal with the problems defined in last year's report from the Warnock committee, but which should also have seen the danger that its plans could be so easily undermined.

What the House of Commons seems not to appreciate is that it has embarked on an unprecedented and dangerous course, the outright banning of certain kinds of research. Its intention, following Mr Powell, is that there should literally never be in Britain even the most sober and well-intentioned investigation of human embryos.

But why should research be sacrosanct, especially when it flatly conflicts with what seems to be an absolute moral principle? This is what the 238 Members of Parliament who voted for the Powell bill were asking two weeks ago. What the scientific community must now recognize is that, while there may be arguments about the force of the moral principle, there is very little chance that this substantial body of opinion in the House of Commons will simply melt away. The practical task is somehow to persuade a large group of sensible people, who must constitutionally be supposed to represent a comparable body of opinion in the general population, that some other course of action than Mr Powell's bill will meet their need.

The starting point must be that research is not sacrosanct. There are already many fields in which investigators do not enjoy untrammelled freedom to do what they like. In most countries, experiments with animals are quite properly regulated with the objective of minimizing the pain that may be suffered in the best-designed experiments. Here the guiding principles are that animals, whose lives are not protected as are those of human beings, must nevertheless not suffer needlessly, which is a goal in its own right but which also protects the investigators concerned from debasement. In genetic manipulation and in work with dangerous pathogens, investigators are free to follow their interests only if they conform with guidelines designed to protect the

safety of other people. In most countries, fetal material (as obtained at abortion) can be used, but only with seemingly consideration for its source. More to the point, even investigations in which people are the experimental subjects are allowed, but only under the strictest criteria, among which pride of place is taken by the doctrine that the subject must understand what is proposed and must appreciate what risks may be involved. The plain fact is that there are already many fields of research which raise difficult moral problems. In no case has it yet been thought necessary to institute an outright ban. Regulation has been thought sufficient.

The reasons why an otherwise sensible legislature has on this occasion departed from precedent must be understood more clearly. Part of the explanation lies in the title of Mr Powell's bill, the "unborn children (protection) bill". Logician that he is, Mr Powell's argument against regulation rather than an outright ban is easy to predict: embryos are people or potential people who, because of their condition, are unable to give their informed consent. The best hope of winning a better piece of legislation is the likelihood that not many members of the British House of Commons will share this extreme position.

Even a superficial reading of the debate on 15 February in the House of Commons will show that the supporters of the bill span as wide a spectrum of opinion as would be expected. Mr Powell's acknowledged "deep and instinctive" revulsion from investigations of human embryos was widely shared, while many participants in the debate volunteered the opinion that "scientists must be stopped from playing God". The missing ingredient in the debate was the clear statement of what exactly are the benefits of investigations with human embryos. That is not surprising, for as yet there have been no such investigations.

The most common misapprehension seems to have been the almost entire lack of an understanding of what may be meant by research with human embryos. Within the scientific community, it is too easy to suppose that the implications are generally understood. But the chances are that, for many purposes, mere observation (with the help of a microscope) would suffice. Given the general interest in the early differentiation of embryos, it might also be necessary to separate early embryos into individual cells, perhaps so as to understand the cir-

cumstances in which totipotency is lost (but those are investigations on which work with other mammalian embryos might suffice). The immunology of the early embryo, and questions of teratogenicity, might involve adjusting the chemical environment of an embryo *in vitro* by the addition of identified substances, many of them naturally occurring. In all kinds of investigations, it would be necessary to be able to dissect an embryo after some fixed period of development, perhaps so as to learn something about its genetic structure. Compared with the more lurid suppositions of what the new opportunities in human embryology constitute — schemes for making literally artificial human beings and the like — this is all dull and almost pedantic stuff. Indeed, except in relation to the technology of *in vitro* fertilization, there seems at present something of a dearth of serious proposals for investigations that would make sense.

Accordingly, those with an interest in these opportunities (and in seeing the debate about the Powell bill take a measured course) are invited to take part in an experiment. The intention is to discover what kinds of investigations with human embryos would, in present circumstances, be carried out. Those willing to participate are asked to send to the editor (in London, in an envelope marked "Embryo Research") a synopsis of a proposed investigation with human embryos, giving a succinct account of the objectives, the reasons for expecting these to be attainable, a brief account of the procedure to be followed (together with an estimate of the numbers of embryos required, controls included) and an explanation why the use of human as distinct from other mammalian embryos is necessary.

Synopses received in the *Nature* office within one month of the date of this issue and judged suitable for the purpose will be sent to a panel of referees composed along the lines recommended by the Warnock committee. Opinions will be solicited from lay people as well as from researchers and physicians. Submissions as such will not be published, but (with the agreement of the authors) the gist of each proposal will appear together with the opinions of all the referees. Those whose submissions are dealt with in this way will be asked to nominate someone (possibly themselves) to receive a copy of *Nature* free for the year ahead. The outcome of this experiment may be to put the Powell bill in perspective. □

Condensed matter and planetary science

First-order phase transitions between amorphous ices

from J.P. Poirier

WHAT seems to be an unprecedented case of a first-order phase transition between amorphous solids is reported on page 76 of this issue. Since it concerns amorphous ices, it has some specific importance for the understanding of icy bodies in the Solar System and for the cryopreservation of biological samples. But it also has general implications for the physics of disorder and for a range of solids that might exhibit transitions between amorphous forms.

Amorphous solids can be obtained by a variety of methods: direct condensation of gases onto a cold plate; quenching of liquids through the glass-transition temperature; and intense irradiation by energetic particles or the impact of high-velocity projectiles. Unlike crystals, amorphous solids are disordered in structure, although not as chaotic as was once thought; they lack crystalline order, but according to the way they are prepared and to the nature of their chemical bonding, different kinds of disorder are found. The physics of ill-condensed matter is an active field of research, in which theoreticians and experimentalists alike are busy describing or predicting the structure of liquids, glasses and all sorts of amorphous solids.

The amorphous ices are of particular interest in two respects. First, they may be present in cometary nuclei, interplanetary dust and ring particles, and in the interior or at the surface of the satellites of the giant planets. Water has at least nine crystalline polymorphic varieties, some of which are metastable far away from their stability field. Second, by the use of cryoprotectants to promote the formation of amorphous rather than crystalline ices, living cells are protected against death caused by the volume expansion that accompanies the normal freezing of liquid water into the common form of ice, ice I. One cannot, therefore, overemphasize the interest of two papers from O. Mishima, L.D. Calvert and E. Whalley of the National Research Council laboratories in Ottawa, Canada. In the first (*Nature* 310, 393; 1984), they report the preparation of amorphous ice by 'melting' crystalline ice I under pressure at temperatures below the glass transition of water; now, on page 76, they report the phase transition between this and another variety of amorphous ice.

The melting point of ice I decreases as pressure increases, but at low temperature and high pressure other crystalline phases become more stable than liquid water or ice I. The melting curve of ice I can, however, be extrapolated in the stability field of ice VI, showing that, in principle, ice I

could 'melt' at 77 K and 10 kbar. By compressing ice under these conditions, Mishima *et al.* obtained an amorphous phase, which persists in metastable state on removal of the pressure. The density of the new phase is consistent with the extrapolated density of water under pressure. At atmospheric pressure and 77 K, the amorphous phase has a density of 1.17; it does not have the same X-ray diffraction pattern as the amorphous phase prepared by quenching water by jet freezing (Mayer, E. & Brüggeller, P. *Nature* 298, 715; 1982). On heating to 117 K it transforms, with heat evolution, to a less dense amorphous phase resembling the form obtained by condensation of vapour onto a cold plate at temperatures above 80 K. There is clearly a rich variety of amorphous structures and their relationships have to be investigated.

In their paper, Mishima *et al.* report that compression of the low-density (0.94) amorphous ice obtained by vapour condensation produces the same high-density phase as they obtained by 'melting' ice I under pressure. The new and interesting

point is that the transition, which occurs sharply at 6 kbar, seems to be the first reported case of a first-order transition between amorphous solids.

The theoretical possibility of phase transitions between amorphous solids or quenched liquids with differing packing structures has been considered by F.H. Stillinger and T.A. Weber (*Science* 225, 983; 1984). At a meeting last year on Ices in the Solar System (see *Nature News and Views* 308, 692; 1984) E. Mayer and R. Pletzer reported that vapour condensation onto a cryoplate provides two varieties of amorphous ice: a low-density porous solid when the flow is supersonic and a glass-like, dense non-porous solid when the flow is broken up by a baffle. Their hypothesis about the structures of these phases implies polymorphism for the amorphous ices. It is not yet clear whether the dense amorphous ice produced by Mishima *et al.* is the same as that of Mayer & Pletzer or that reported to be formed by condensation of vapour on a plate at temperatures lower than 10 K (Narten, A.H., Venkatesh, C.G. & Rice S.A. *J. chem. Phys.* 64, 1106; 1976).

Clearly the evidence for first-order phase transitions between amorphous structures opens exciting new vistas in the physics of disorder, although one may, perhaps, shudder at the thought of the pressure/temperature phase diagram of water, with its superposition of many stable and metastable crystalline phases, being further complicated by the addition of boundaries

Riddle of reflections in the water

WHAT is the shape of the image of the mast of a sailing ship seen reflected in the water? Suppose the surface of the water is wavy, but not to the extent of creating sharp wave-crests (that is, the gradient is nowhere discontinuous). Of course, you will say, the image of the mast will be 'wiggly'. But will there be pieces of mast seen disconnected from the wiggly main image? Presumably yes, since there is no reason why there should be not be an occasional area of water surface off to one side and so inclined that it would throw light from the mast to the observer, and at the same time there might be no area in between that would do likewise.

What shape will such a disconnected piece of the mast image have? In particular, will there be 'ends', where the mast image appears broken? The picture on the cover of this week's *Nature* gives the answer. Disconnected pieces are closed loops. Why?

Here is a simple way to visualize the situation. Suppose instead of the mast we have a vertical line dividing a black area on the left from a white area on the right, each such area stretching out indefinitely far to left and right respectively, as well as upwards. The image area in the water on the right-hand side will then be largely white, but it may contain some

black patches. Again, it is clear that they can be disconnected, meaning that they are wholly surrounded by white, since there can be an occasional patch of water surface at such an inclination that it looks into the black side of the object plane. What is the nature of the edge of such a black patch?

We may choose two closely adjacent points in the image, one within the black patch and the other just outside, in the white. Since the water surface has no discontinuities of gradient, the two adjacent rays must come to adjacent points in the object plane. But the only places where black and white areas are adjacent is on the dividing line. Since this consideration applies to every pair of points straddling the edge of our black patch, it implies that its entire edge must image a part of the dividing line into a closed loop, wherever a disconnected patch occurs. The appearance of the sailing ship mast follows from the same considerations.

There is one additional condition required for all this to be true. The surface of the water must not anywhere be inclined to the horizontal as steeply as the line of sight from the observer, so no area of the surface is shielded from view.

Thomas Gold, Cornell University.

between metastable amorphous phases. The consequences extend further, as Mishima, Calvert and Whalley point out: other solids, which melt with a decrease of volume (like germanium, silicon and boron nitride), might exhibit several amorphous structures, some of them possibly resulting from 'melting' under pressure at low temperature.

Water vapour in the outer reaches of the Solar System that is condensed as amorphous ice and small bodies, like the particles in the rings of Saturn, the nuclei of comets and small icy satellites, may still contain a large proportion of (presumably) low-density amorphous ice (Smoluchowski, R. *Science* **222**, 161; 1983). Meteoritic impacts on the surface of icy bodies may also form amorphous ices by direct amorphization due to the shock, reversion of shock-produced high-pressure phases, or

recondensation of vapourized water. It has been suggested that the heat evolved during the transition from amorphous ice to cubic ice I at 153 K provides the supplement of energy needed to melt and resurface Saturn's satellites Enceladus and Dion (Klinger, J. *Nature* **299**, 41; 1982) and alters the heat and mass balance of comets (Klinger, J. *Science* **209**, 271; 1980). As pointed out by Mishima *et al.*, we now have to take into consideration the possibility that low-density amorphous ice might transform into dense amorphous ice during accretion of the larger icy satellites (like Ganymede, Callisto or Titan) and then re-transform exothermally into a variety of amorphous structures as the temperature increases inside the planet. □

J.P. Poirier is at the Institut de Physique du Globe de Paris, 4 Place Jussieu, 75230 Paris Cedex 05, France.

Evolutionary biology

A new phylogeny for Darwin's Galapagos finches

from Paul H. Harvey

EVOLUTIONARY biologists owe it to the memory of Charles Darwin to produce a phylogeny of his famous Galapagos finches. Schluter has produced one such phylogeny in a recent paper¹; more importantly, he constructs it using a new method which assumes that natural selection alone has caused changes in morphology among the different finch species. Darwin might have appreciated that.

There have been two previous attempts to describe the relationships among the various finch species. The first, by Lack², was a qualitative assessment based on comparative morphology. The second, by Yang and Patton³, used electrophoretic variation of proteins. Differences between the two estimates may result both from the different databases used and the failure to base the analyses on suitable models of evolutionary change.

The justification for Schluter's new analysis comes from recent ecological studies on Darwin's finches by Grant and his colleagues^{4,5,6}. Their work leads to the conclusion that morphological variation among the finches is largely the result of differentiation by natural selection. But genetic constraints must also be taken into account if an evolutionary tree is to be derived on the assumption that differences between species are purely a result of natural selection. Genetic variances and covariances among the characters used in the analysis are the two relevant genetic constraints⁷. If genetic variances are high then characters are more susceptible to change by natural selection. If two characters covary positively then more selection is required to increase one and decrease the other than if the genetic

covariance were to be negative. Lande⁸ produced a quantitative genetic model that takes these factors into account and Schluter has adapted the model in order to estimate the total net force of natural selection that has led to differentiation among Darwin's finches. His analysis is based on phenotypic variances and covariances among eight measures of wing, tarsus and mandible size. The variances and covariances among the characters are similar within ground finches and within tree finches but differ between the two groups, so analyses were performed separately.

Phenotypic measures do not necessarily reflect underlying genetic trends but, fortunately, it appears that they do in the case of Darwin's finches. A recent genetic analysis of variation in the tree finch *Geospiza fortis*, which investigates five of the eight characters used by Schluter, shows that the five phenotypic and genetic variances correlate with a value of 0.98, while the ten covariances have a 0.99 correlation⁹. This allowed Schluter to substitute phenotypic estimates for the genetic variances and covariances.

Schluter includes the warbler finch *Certhidea olivacea* in both the ground finch and the tree

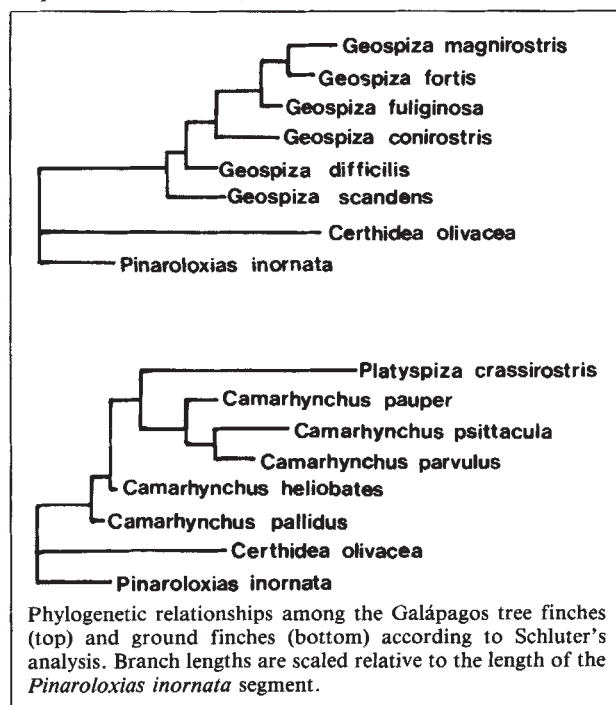
finch analyses because this species probably branches off prior to the division of the two groups; its omission from the calculations does not change either of the phylogenetic trees. Like Lack before him, Schluter incorporates data from Cocos Island's putative warbler finch *Pinaroloxias inornata*.

The new evolutionary trees, shown in the figure, are similar to Lack's, but there are some differences; the ground finches *Geospiza difficilis* and *G. scandens* appear more closely related than in Lack's analysis, while the tree finches *Camarhynchus pallidus* and *C. heliobates* come close to the ancestral condition according to Schluter, yet are highly modified forms according to Lack. Yang and Patton's electrophoretic analysis resulted in a tree similar to Schluter's, although it is based on only eleven of the fourteen species used by Schluter and Lack.

The new method differs from standard cladistic techniques¹⁰ in measuring branch lengths of the phylogenetic trees, while quantitative variation in the original characters is more effectively incorporated into the analysis. Equally important is the use of distance measures which are based on an explicit genetic model, many of the assumptions of which seem reasonable in the present case. □

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Paul H. Harvey is in the Biology Department, Princeton University, Princeton, New Jersey 08540, USA.



Phylogenetic relationships among the Galapagos tree finches (top) and ground finches (bottom) according to Schluter's analysis. Branch lengths are scaled relative to the length of the *Pinaroloxias inornata* segment.

Stellar structure

Beginnings of asteroseismology

from Douglas Gough

THE science of determining the internal structure of stars from the properties of dynamical oscillations, known as asteroseismology, has only recently become a possibility. Solar five-minute oscillations^{1,2} have already yielded important information about the structure and composition of the Sun. Now similar five-minute oscillations have been reported for three distant stars³⁻⁵. Interpretation of the data from two of these^{3,4} provides few surprises, but the third⁵ seems to be anomalous and may pose a new and interesting problem to stellar astronomers.

The significant breakthrough came when Isaak *et al.*² discovered that they could detect the five-minute oscillations from the Sun in spatially unresolved whole-disk Doppler data. Some spatially resolved data¹, available for several years, had convincingly demonstrated that the five-minute oscillations are a manifestation of resonant acoustic waves trapped beneath the solar photosphere. Although important conclusions about the structure of the Sun's convection zone have been drawn from these observations, such detailed information cannot be obtained from other stars. Whole-disk observations, on the other hand, use light integrated from the entire visible surface of the Sun, which is just what one is forced to do when observing the distant stars.

Oscillations of ordinary stars have the spatial variation of spherical harmonics. For each value of the degree l of the harmonic, there is a sequence of resonant acoustic modes that are labelled with the integer n . These correspond to the fundamental tone ($n = 1$) and the overtones (of order $n - 1$) of a musical instrument. On the whole, n is the number of spherical nodal surfaces inside the star, and is a measure of the vertical component of the wave number of the acoustic wave; the horizontal component of the wave number is approximately l/r , where r is the distance from the centre of the star.

A resonant acoustic mode can be considered as an appropriate superposition of many propagating sound waves with the same frequency. Because temperature, and hence sound speed, increase with depth inside a star, downward-propagating waves are refracted back towards the surface. Waves with periods longer than about three minutes, in the case of the Sun, are also reflected by the highly stratified material immediately beneath the photosphere. When l/n is large, the waves are refracted quickly, and are thus confined to a thin outer shell immediately beneath the stellar surface. Its thickness is about $(2n + 3) R/l$, where R is the radius of the star. When l/n is small, the waves propagate nearly ver-

tically, and penetrate to the core of the star. For the Sun, it has been possible to infer the variation of the sound speed through much of the interior⁶ by analysing five-minute oscillations with a wide range of l/n , with a technique bearing some resemblance to that used by particle physicists to infer potentials from scattering experiments.

Whole-disk observations are sensitive to only the lowest values (0-3) of l , and consequently cannot access a very wide range of l/n . Nevertheless, in principle one can still gain useful information about the stellar interior. The density and pressure throughout the star can be expressed in terms of linear combinations of oscillation eigenfunctions, with coefficients that depend in a calculable way on the eigenfrequencies, rather like expressing a function as a Fourier series. By measuring oscillation frequencies one might therefore hope to determine how the star is stratified. Indeed, using artificial data it has been demonstrated that if the frequencies of a substantial number of appropriate low-degree solar oscillations are known, the interior pressure and density distribution can be inferred quite accurately, provided the variation of the adiabatic exponent is unimportant.

Unfortunately real data of that kind are not yet available. In the case of the Sun, the acoustic modes are excited to an amplitude great enough to have been detected only in a frequency range between about 2 mHz and 4 mHz (though there is now evidence that another class of modes—the gravity modes, whose restoring force is provided by buoyancy—has also been observed). These modes are quite high overtones, with n between approximately 12 and 33, and by themselves are inadequate for describing the interior stratification—without the lowest sinusoids in a Fourier expansion one cannot in general obtain a good representation of a function. Nevertheless, one can draw some useful conclusions.

The cyclic frequencies ν of low-degree acoustic modes satisfy

$$\nu = (n + \frac{1}{2}l + \alpha)\nu_0 + \varepsilon(n, l),$$

where α and ν_0 are constants and $\varepsilon/\nu = 0$ (n^{-2}) when n is large. With the exception of only the lowest-order modes, the frequencies form groups with alternate odd and even l , which are approximately uniformly separated by $\Delta\nu = \frac{1}{2}\nu_0$. A moderately resolved power spectrum of oscillation data should reveal this spacing. It establishes the value of the base frequency ν_0 , which is half the reciprocal of the time taken for sound to travel from the star's centre to its surface.

The whole-disk observations first

reported by the Birmingham helioseismologists² clearly exhibit this property in the oscillations of the Sun, with $\Delta\nu$ of about 67.8 μ Hz. Now we have the first indications of similar oscillations in other stars. By combining photometric observations of the Ap star HR 1217 over the same time period from South Africa and Chile, Kurtz and Seeman³ have found six approximately uniformly spaced frequencies near 3 mHz. Fossat *et al.*⁵ and Noyes *et al.*⁴ also have evidence for uniformly spaced frequencies of α Centauri A and ε Eridani, respectively.

The mean frequency separation measured by Kurtz and Seeman is 34.7 μ Hz. As Shibahashi⁷ has pointed out, the situation is likely to be similar to that of the Sun: modes of both odd and even degree are probably present, so the observed spacing $\Delta\nu$ approximates $\frac{1}{2}\nu_0$. Computations by Christensen-Dalsgaard⁸ show that on the zero-age main-sequence, $R\Delta\nu$ is roughly constant. (Main-sequence stars, like the Sun, are at the stage of their evolution where they are powered by hydrogen fusion in their cores). In other words, all zero-age main-sequence stars have the same harmonic mean sound speed. If evolutionary effects are small we can deduce that HR 1217 has about twice the radius of the Sun, a value typical of an Ap star.

The observations of α Centauri A and ε Eridani are particularly interesting because these stars are quite similar to the Sun. Their oscillation amplitudes are much smaller than those of HR 1217, so more sensitive observing techniques were required. Fossat *et al.*⁵ observed variations in the intensities at fixed frequencies on the red sides of the Na-D spectrum lines, with the intention of detecting Doppler shifts. Noyes *et al.*⁴ observed variations in the profiles of the Ca II-H and -K chromospheric emission lines. Both found evidence for discrete oscillatory modes. After a certain degree of filtering, it was deduced that the envelope of the power spectrum for α Centauri has a broad hump centred at about 3 mHz, like that for the Sun. In neither case was the power spectrum clean enough to identify individual modes unambiguously, so there is room to doubt the interpretation of the data. Nevertheless, the power spectrum of the power spectrum for α Centauri A⁵ and the autocorrelation of the power spectrum for ε Eridani⁴ both suggest a periodicity in the power, at frequency separations of 81.3 μ Hz and 86 μ Hz, respectively.

The approximate constancy of $R\Delta\nu$ found by Christensen-Dalsgaard⁸ for the zero-age main sequence is also a property of main-sequence stars with the same evolutionary age, though the value of the constant changes as the stars evolve. The homologous scaling $R^{3/2}\Delta\nu = \text{constant}$ is approximately satisfied by a star during its central hydrogen-burning phase, even though the changes the star undergoes are not strictly homologous.

As pointed out by Noyes *et al.*⁴, their observations of ε Eridani appear to be con-

sistent with these scaling laws. Judged by its strong Ca II emission, the star seems to be younger than the Sun, and Lacy⁹ has established that $R/R_{\odot} = 0.81 \pm 0.08$, where $R_{\odot}$ is the present radius of the Sun. Since $R\Delta\nu/R_{\odot}$ is calculated to be $72.5 \mu\text{Hz}$ for the zero-age main sequence, and $\Delta\nu$ is about $68 \mu\text{Hz}$ for the Sun now, one would expect $\Delta\nu$ for ϵ Eridani to be $84\text{--}90 \mu\text{Hz}$. This is indeed the case.

The observations of α Centauri A do not conform to this pattern. The star is a member of a binary system and is known to have a mass of $1.1 M_{\odot}$. According to Christensen-Dalsgaard and Frandsen¹⁰, $\Delta\nu$ should have been $71 \mu\text{Hz}$ when such a star was on the zero-age main sequence; its radius should have been $1.03 R_{\odot}$. Since $\Delta\nu$ is now $81.3 \mu\text{Hz}$, one would deduce from homologous scaling that R is approximately $0.94 R_{\odot}$. Having a higher mass and apparently a lower radius than the Sun, at first sight α Centauri might therefore also be thought to be younger because, according to the theory of stellar evolution, the radii of late-type main-sequence stars increase with both mass and age. But the observed $\Delta\nu$ appears to imply that α Centauri A has shrunk. This contradicts observation as well as theory, since Blackwell and Shallis¹¹ find from the infrared magnitude and a knowledge of the distance of α Centauri that $R/R_{\odot} = 1.23 \pm 0.04$. Moreover, Flannery and Ayres¹² conclude, from matching the theory of the evolution of the companions of the binary system with all the available observations, that α Centauri is probably older than the Sun. Either the oscillation data have been misinterpreted or the structure of α Centauri A is quite different from expectations.

More highly resolved measurements of the oscillations will have to be made in order to decide between these possibilities. It would help to have several days of almost continuous data, which can be obtained by observing from two or more sites widely separated in longitude over the same period, as has already been done for HR 1217 and the Sun^{3,13}. Alternatively the stars might be observed from Antarctica or from space. From such data, it may also be possible to measure the small quantity ϵ in the dispersion relation, which separates the frequencies of modes with like $n + \frac{1}{2}l$ but different l , and which measures conditions close to the centre of the star¹⁴. This would give us direct information about the stratification of the energy-generating core of a distant star, an exciting prospect indeed. $\square$

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Douglas Gough is a visitor at the Tata Institute of Fundamental Research, Homi Bhabha Road, Bombay, India. His permanent address is Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, UK.

Scrapie

Perspectives on prions

from Colin Masters

AFTER decades of research into a group of 'slow virus' diseases, Stanley Prusiner and his collaborators have published a continuing series of papers identifying a new type of infectious particle—the prion (for proteinaceous infectious particles). The remarkable resistance of the prion to chemical and physical procedures that attack nucleic acids, and its small size (reportedly 100 times smaller than the smallest virus) have directed attention towards the possibility that the prion consists of protein without associated nucleic acid and that its replication proceeds via protein-directed protein synthesis—a situation which would be without precedent in biological science and contrary to the dogma that genetic information invariably flows from nucleic acids to proteins. Consequently, it is not surprising that the research (see refs 1 and 2 for reviews) has given rise to a great deal of speculation on the nature of prions, on the alternative

possibilities of their replication, and on their significance in relation to several chronic degenerative diseases of humans. The potential ramifications make it worth assessing the status of prion research.

The research may be traced back to the 1950s when the first intensive studies began in the highlands of New Guinea into the strange degenerative condition known as kuru³. The dramatic nature of these findings (kuru was found to be transmitted by the cannibalistic habit of eating uncooked brain) stimulated interest in scrapie, a related disease of sheep and goats which can be produced experimentally in small laboratory animals. Scrapie researchers have coped with many formidable technical difficulties but after experimental models of scrapie were produced in mice and hamsters in the 1960s, several groups began to purify the causative agent^{2,4,5}, which was presumed to be a virus. It became apparent that the agent was firmly associated with

cell membranes and could only be released by quite drastic treatment. Proteases, detergents and denaturants were commonly used in purification and it was the remarkable stability of the infectious agent to these substances, together with its resistance to nucleases and ultraviolet irradiation that led to a view that the scrapie agent may not be a virus at all.

Much of the data pointed to the importance of the protein content of the particle for scrapie infectivity. This, together with the continued failure to demonstrate the existence of a scrapie nucleic acid, led to the suggestion that the protein may either code directly for its own replicative synthesis or induce genes of the infected animal to do so for it¹. Subsequently, similarities between prion aggregates in tissues and the amyloid plaques that are features of several human diseases were pointed out by Prusiner, who suggested the involvement of prions in a wide range of conditions including senile dementia, multiple sclerosis, rheumatoid arthritis, Parkinson's disease, diabetes, lupus erythematosus and cancer². Clearly, if these postulates are substantiated, they will have momentous implications for biomedical science.

What remains to be done then before this critical and highly contentious situation is finally clarified? At present, quite severe criticisms of major elements of the prion hypothesis are still appearing in the scientific literature^{6,7}. Several authors have pointed to the fact that much of the evidence is negative in nature—never a fully satisfying situation for the scientist. Much of the data on the physiochemical characteristics of the infectious agents in tissues is difficult to interpret because of the crudity of the extracts. It has been suggested, for example, that the organism's extraordinary resistance to inactivation, which has led to much of the present speculation on this new life form, may not be a feature of the overall population of particles but rather is limited to small sub-populations. It has, moreover, been claimed that when conventional viruses are suspended in tissue homogenates, they too can provide examples of resistant populations and insensitivity to virucidal agents. The deduction that the scrapie agent has a sub-viral size has also been questioned, and it has been pointed out that there is good *a priori* evidence that the scrapie agent has a nucleic acid genome, even if direct biochemical evidence is lacking. Finally, it has been argued that if all the scrapie agent has to do is be replicated by host enzymes and interact with the host in strain-specific ways to produce disease, its nucleic acid could be unusually small and difficult to detect.

Clearly, it would be possible to resolve most of the outstanding questions if the infectious agent were available for examination in purified form. It is, therefore, encouraging to note that rapid progress is being made in that direction. It

has been established that the bulk of the protein in the scrapie agent consists of a single molecular species (called PrP, for prion protein)⁸. PrP is a glycoprotein, of apparent relative molecular mass of 30,000, and has been highly purified and partially sequenced. When the complete sequence has been determined, it will be possible to synthesize the same sequence and test whether it has the same biological action as the natural agent. Only then will it be determined with certainty whether prions are both infectious and devoid of nucleic acids. When the synthetic molecule is available, antibodies to it could also be produced for titration of scrapie, and synthetic sequences of the corresponding gene could be constructed and used to investigate both the function of the PrP gene in the cell and the presence of the PrP gene in the host genome. It will also be important to determine the amino-acid sequences for myeloid proteins and compare them with PrP to establish the exact connection between amyloid plaques and the infectious agent.

The imminent resolution of many of these questions heralds an exciting era of biomedical advance. An intriguing area of

scientific curiosity which has been impeded by forbidding technical problems has been transformed into a rapidly progressing and productive area of contemporary research. Already, the immunological evidence available appears to provide support for the view that the amyloid plaques in the brains of scrapie-infected animals and humans with Creutzfeldt-Jakob disease are composed of arrays of prion proteins⁹. Soon we should know for certain whether prions do indeed represent a revolution in molecular biology and medicine, or if they are another manifestation of the complexity of interpreting molecular behaviour in the cellular microenvironment. □

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Colin Masters is in the School of Science, Griffith University, Nathan, Brisbane, Queensland, Australia 4111.

Immunology

Control of T-cell development

from Roland Scollay

OUR understanding of the control of the proliferation and early differentiation of T lymphocytes in the thymus gland is hazy, to say the least. Yet the thymus supports a large population of dividing cells¹ and about 30 per cent of its lymphocytes are replaced every day². The hope that either one of the thymic hormones or interleukin-2 (IL-2) — a potent growth factor for mature stimulated T lymphocytes — would turn out to be the key factor for proliferation of the blast cells from which T lymphocytes are derived, has not been realized^{3,4}. As a result the notion has arisen that growth factors are less important for proliferation than the interaction between blast cells and other non-lymphoid cells of the thymus. But two papers in this issue^{5,6}, showing IL-2 receptors to be on a subset of cells in the thymus, come as a faint ray of hope that IL-2 is the key.

About 80 per cent of the blast cells (15 per cent of all cells) in the murine thymus are the parents of the major population of small thymocytes in the cortex of the thymus. On their cell surfaces, they carry two antibody-defined markers associated with functional T lymphocytes⁷. The markers are Ly2 and L3T4, so the cells are classed 2⁺4⁺. Most of the other blast cells (about 3–5 per cent of all thymocytes) are 2⁺4[−] — that is they express neither Ly2 nor L3T4 and are generally felt to be precursors of, presumably, both cortical and medullary cells⁷. These 2⁺4[−] blasts are the predominant population in the embryonic murine

thymus from day 12–16 or 17 of gestation, an observation which lends credence to their role as precursors⁸.

It is on these 2⁺4[−] blasts that receptors for IL-2 have now been demonstrated. Ceredig *et al.*⁵ use an antibody to the IL-2 receptors to show that about half the 2⁺4[−] blasts in both embryonic and adult thymus express the receptor, although those from the adult thymus (embryonic not tested), are slightly fewer in number and lower in affinity than those found on mature lymphocyte (Con A) blasts. They also demonstrate that the cells with IL-2 receptor are scattered throughout both the cortex and medulla of the thymus. Using different monoclonal antibodies, Raulet⁶ finds a very similar distribution of the receptor on adult and embryonic blasts but, unlike mature lymphoid blasts which respond vigorously to IL-2, neither population of thymic blasts responds to IL-2 unless an additional stimulus (Con A) is added.

These data are relevant to a number of issues. First, it has generally been felt that 2⁺4[−] blasts are mainly confined to the outer cortex of the thymus, even though blasts in general, although more concentrated in the outer cortex, are widely distributed. The data from Ceredig *et al.*⁵ show that the IL-2 receptor-positive 2⁺4[−] blasts are also widely distributed. Whether 2⁺4[−] cells occur in the medulla is still unclear, since it has not been established whether all the IL-2 receptor-positive cells are 2⁺4[−], as both papers point out.

The second but related point concerns the heterogeneity of 2⁺4[−] cells. Other work shows that they are not a homogeneous population. Are receptor-positive and receptor-negative cells precursors for two pathways or lineages, or are they sequential stages of one (or more) pathway? The new data open up several interesting lines of experiment to answer such questions.

The third issue concerns the role of the receptor in cell differentiation. The receptors on thymic 2⁺4[−] blasts have relatively low affinity for IL-2, which perhaps accounts for the fact that the cells are not well stimulated by IL-2 alone. Unfortunately, there is no affinity data for the embryonic cells, which respond even less well to IL-2 alone, so it remains unclear whether this reflects a real difference in receptors or a response by the unskilled mature cells in the adult 2⁺4[−] preparation.

Nonetheless, the presence of the receptor in the embryo is important in interpreting the adult data. In general, immunologists associate IL-2 receptors with antigen- (or mitogen-) activated T lymphocytes and with mature cells that produce IL-2. The fact that before day 16 the embryo seems not to express antigen receptors⁹ and that neither IL-2 producing cells nor cells of the usual (mature) IL-2 producing phenotype are detectable until several days later⁸, suggests that the receptor on 2⁺4[−] cells may be operating quite differently from those on mature T cells. Indeed, the whole process within the thymus must be quite different, since the major, rapidly-dividing 2⁺4⁺ blast population, which presumably derives from the 2⁺4[−] blasts, is completely lacking the receptor^{5,6} unlike any other population of activated lymphocytes. Perhaps we should not be unduly influenced by our knowledge of the activation process in mature cells in considering the significance of the IL-2 receptor on thymic cells and its presence on cells should not necessarily be taken as evidence that they are stimulated by antigen.

Some evidence of IL-2 production in the embryo would go far to establishing the relevance of the receptor. Without this, I find it too early to speculate on the receptor's role. But clearly, these experiments and the rapid increase in knowledge of the T-cell receptor brings us closer to an understanding of the secret workings of the thymus. One day a cocktail of factors added to a single T-cell precursor may allow us to observe the complete process of T-cell development in a test tube; but it may yet turn out that cell-to-cell interactions are essential. □

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Roland Scollay is at the Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria 3050, Australia.

Periodic extinctions

Cratering theories bombarded

from Paul Weissman

ONE of the first lessons for any young scientist is how to limit research to a finite task. "All of us", wrote geophysicist E. C. Bullard in 1960, "dig our own small specialized holes and sit in them". For those scientists who venture out of their holes, few problems today are so quick to bring on feelings of agoraphobia as the study of biological extinctions of species on the Earth. Once relatively confined to the planet, the various aspects of the extinction problem have reached out first into the Solar System, and now to the Galaxy surrounding it. To explore these new horizons, astronomers and other concerned scientists met in January* to examine, and heatedly debate, the possible interactions between the Earth, the Solar System and the Galaxy.

The problem of biological extinctions was once the sole province of the palaeontologists and biologists who studied the fossil record of the rise and fall of species. Then, in 1980, physicist Luis Alvarez and his geologist son, Walter, along with colleagues, suggested that the Cretaceous-Tertiary extinction 65 Myr ago was associated with the impact of a 10 km diameter asteroid. They found a strong concentration of iridium in the boundary clay coincident with the extinction. Iridium is very rare in the Earth's crust, most of it having gone into the core when the planet differentiated.

A second expansion of interest in extinctions has followed last year's report by David Raup and John Sepkoski that the extinctions of marine animals over the past 230 Myr seems to have a 26 Myr periodicity. This sparked a number of hypotheses for generating periodic showers of comets from the Sun's Oort cloud — 10^{12} – 10^{14} comets surrounding the Solar System and extending out to interstellar distances. Other hypotheses involved a possible association of extinctions with the Sun's harmonic motion above and below the galactic plane, which has a half-period of 33 Myr. Analysis also indicated a possible 28 Myr period in the record of large, well-dated impact craters on the Earth.

One of the first items called into question at Tucson was the reality of the 26–28 Myr periods in the fossil and crater records. Michael Rampino and Richard Stothers (Goddard Institute for Space Studies) reported a variety of periods, ranging from 12–36 Myr, from a reanalysis of the data, and similar periods in episodes of low sea levels on the Earth, geomagnetic reversals, and major tectonic episodes. They suggested that the periods are all harmonics of the Sun's 33 Myr interval between galactic-plane crossings and that a weakly-

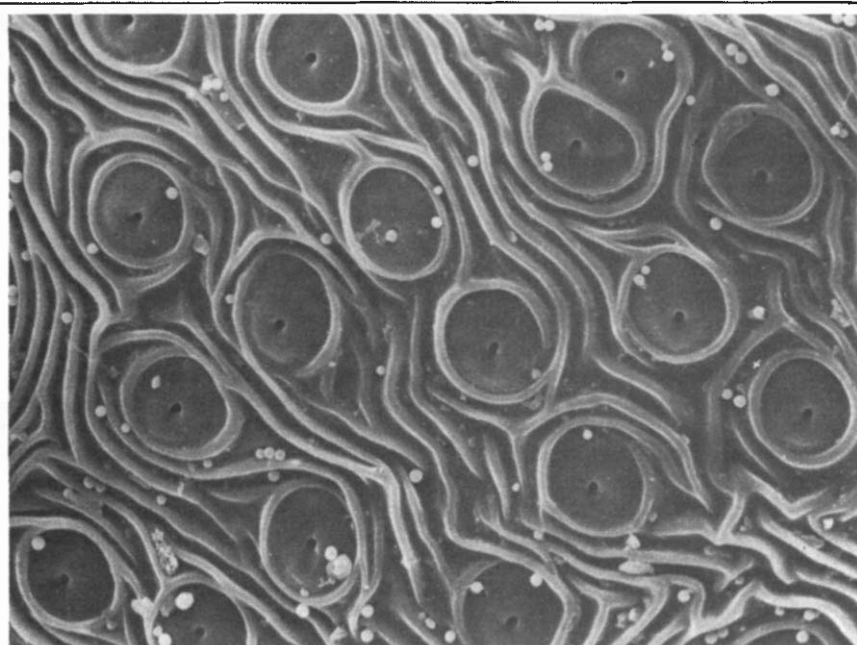
detected period of about 250 Myr is associated with the mean time between the Sun passing through galactic spiral arms.

Critics of these analyses pointed out that Rampino and Stothers had not identified any mechanisms for relating the various geophysical events to the Sun's z-motion. It has been suggested that the probability of the Sun encountering a galactic giant molecular cloud increases sharply as the Sun passes through the galactic plane, every 33 Myr. But Patrick Thaddeus and Gary Chanan (Goddard Institute for Space Studies) showed that such clouds were well dispersed above and below the galactic plane, and that encounters would occur at essentially random times (see page 73 of this issue). In addition, Piet Hut (Institute for Advanced Study, Princeton) and Scott Tremaine (MIT) presented a detailed analysis showing that galactic giant molecular clouds are not likely to be major perturbers of the Oort cloud, contrary to the idea of Biermann and Lust in 1978 and strongly argued for ever since by Victor Clube (University of Oxford) and William Napier (Edinburgh Observatory).

Gene Shoemaker (US Geological Survey) reported a reanalysis of the cratering and extinction records, with a best fit period of 31–32 Myr. But he pointed out that the dating for many of the extinction events is extremely poor, especially beyond about

140 Myr ago. Shoemaker also noted that because most workers in this field now accept the idea that impacts and extinctions are related, the fossil and crater records are not independent, and might well agree in both period and phase of any pseudo-periodicity. As compelling as the evidence may seem, Shoemaker characterized it as 'shot noise' — simply a sequence of very few random events arranged with apparent periodic spacing. Support for this view came from Tremaine, who generated several hypothetical histories of random cratering and then challenged the audience to distinguish the real history from the 'best' random sample. About one-fifth of the assembled scientists chose the random sample. Tremaine then showed that statistical analysis in the random data gave a better fit to the 26-Myr period than the actual cratering history.

All this did not deter the proponents of the several mechanisms for creating a 26-Myr periodicity in showers of comets from the Oort cloud. The idea of an unseen solar companion star with a 26-Myr period has been suggested independently by Daniel Whitmire (University of Southwestern Louisiana) and Albert Jackson (Computer Sciences Corporation), by Marc Davis and Richard Muller (University of California, Berkeley), and by Piet Hut. The unseen star, presumably of low mass, would have to be in an orbit with a semi-major axis of about 87,000 astronomical units (1.4 light years) to have a 26-Myr period, but with a high eccentricity, of 0.7 or more, so that the perihelion distance is close to the inner Oort cloud where the comet density is highest. Dynamical studies by Hut, Mark



Pustuliform organs on the abdomen of the spider *Holcolaetis vidua* Lessert, magnified $\times 880$. It may be that pheromones are secreted through the pore in each organ, and it is not inconceivable that the spherical bodies associated with, or even lodged in, the pores are 'packets' of pheromones. Mytiliform organs on the abdomens of *Spartaeinae* spiders may serve the same function. The two types of organ are described and compared by F.R. Wanless in the course of a revision of the spider genus *Cyrba* in the Bulletin of the British Museum (Natural History), Zoology Series 47, 445 (1984).

*'The Galaxy and the Solar System', Tucson, Arizona, 10–12 January 1985.

Bailey (University of Manchester), and Jack Hills (Los Alamos) showed that such an orbit was unstable and would be likely to escape from the Solar System in less than 1.0 Gyr. Since capture of a companion star is extremely unlikely, the orbit must have been more tightly bound in the past.

The Berkeley group believes that the last periodic extinction was about 12–14 Myr ago, so the proposed companion star, which they call Nemesis, is now near aphelion. Muller described an optical survey of 5,000 low-mass red dwarf stars, which is expected to examine all candidate stars visible from the Northern Hemisphere by the end of this year, and will be extended to the Southern Hemisphere in 1986. Nemesis, if it exists, might also be detected by the all-sky survey of the Extreme Ultraviolet Explorer Satellite, scheduled to be launched in late 1988.

Whitmire and John Matese (University of Southwestern Louisiana) suggested an alternative mechanism for creating periodic cometary showers that involves a tenth planet, some 100–150 AU from the Sun. They suggest that the orbit of the planet is eccentric and highly inclined, and that it has swept a 'clear zone' out of the inner ring of the Oort cometary cloud. As the orbit precesses, however, it will occasionally come close to the edges of the clear zone and again perturb the comets there. The comets are first thrown into Jupiter-crossing orbits, and then some fraction of them reach the Earth's orbit. Comets which do not hit the Earth are eliminated by Jupiter's perturbations in a period of about 1 Myr. Whitmire and Matese believe that the tenth planet has a mass one and a half times that of the Earth and an orbit inclination of at least 45 degrees.

I raised a number of objections to the hypotheses involving cometary showers. Current estimates of the cratering rate on the Earth and Moon from known craters on dated surfaces agree well with expectations from the observed flux of Earth-crossing comets and asteroids, whereas adding intense comet showers every 26 Myr would raise the cratering rate by a factor of 5–18, inconsistent with the observed craters. The only way out of this dilemma is to assume that the Solar System is currently in the midst of a cometary shower, and that the 'steady state' observed cometary and asteroid flux (many dynamicists hold that the Earth-crossing asteroids are extinct comets) is anomalously high. This might work with the 31–33 Myr period, which puts the last periodic extinction only 4–5 Myr ago, but not with the 26 Myr period, which sets the last extinction some 10 Myr farther back in time. Moreover, analyses of impact melts from terrestrial craters suggest that many were made by highly differentiated meteorites, like irons and achondrites (similar to basalts). This is inconsistent with a cometary source, since comets are believed to be the most pristine undifferentiated objects in the Solar System, a conclusion supported by analyses

of interplanetary dust collected by high-flying aircraft.

The advocates of cometary showers countered that the identifications of the objects that formed the crater are still very tentative, and that the flux of comets in a shower might be up to an order of magnitude less than their original estimates, thus lowering the predicted cratering to acceptable levels. The question of whether the lower fluxes would also decrease the probability of major biological extinctions was not answered.

In the end, no hypothesis for or against periodicity emerged as clear winner, and

few converts were made, but a very convincing picture emerged of the ways in which the Galaxy could affect the environment within the Solar System and on Earth. Although the conference participants did not come to any clear consensus on the extinction problem, they returned home with broadened horizons and, perhaps, with the temptation to continue to explore beyond their specialized holes. □

Paul Weissman is in the Earth and Space Sciences Division, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California 91109, USA.

Biogeology

Sulphur-eating tube worms take to the Oregon breaches

from Roger N. Anderson

SCIENTISTS attending meetings of the American Geophysical Union (AGU) have grown accustomed to new reports of under-sea oases of brilliant red tube worms and giant red-meated clams clustered around black smokers, which billow forth 400°C hydrothermal fluids at the axes of mid-ocean ridges. New communities from the eastern Pacific have been reported with remarkable regularity since their initial discovery in 1977. More were reported at the AGU winter meetings in San Francisco (4–9 December 1984), but there was a big surprise in store. A series of four consecutive papers^{1–4} documented the presence of sulphur-eating tube worms and giant clams in a completely new environment: on the accretionary pile of sediments found above a subduction zone, where there are no hot springs.

The biological communities were found living along 'breaches' or open rifts that were discovered during dives of the submersible *Alvin* along a series of anticlinal folds of newly accreted sediments on the continental margin off Oregon, in the north-west United States. No hot-water springs were detected by the sensitive thermometers on *Alvin*, but methane-enriched waters were captured. These, along with methane-derived calcium carbonate cementation found within the surface sediments, verify that sulphur-rich waters are being expelled along the breaches. Methane and sulphur are both added to hydrothermal waters during chemical interaction with basalts of the oceanic crust.

Though not associated with hot springs, the methane-rich waters of the breaches probably have a source similar to that at ridge crests. The subduction of the Juan de Fuca plate under the coast of Oregon is the likely source of the sulphur and methane. Waters circulating in the crust eventually end up as pore fluids in sediments being accreted to the west coast of Oregon. Dewatering of these sediments is apparently

a more dominant physical process than previously thought, since sufficient flow must be occurring to supply enough nutrients to support the same kind of biological community as that living near ridge-axis black smokers. Contrary to popular belief, these new observations show that hot temperatures are not required to support the organisms.

The typical sedimentary diagenesis that is observed in the area points to widespread venting all along this margin. The microcrystalline dolomite, magnesian calcite, and aragonite that surround the vents result from methane reactions. Such mineralogy is common throughout older accretionary complexes of the world. Dewatering of 20 per cent by volume of the accretionary sediments is required to support the volume of diagenetic sediments observed off Oregon. The dewatering becomes more pronounced as one proceeds away from the trench axis toward the shoreline. Tube-worm communities were sampled on vents in sediments accreted up to 1.8 million years ago.

These discoveries, and another report⁵ of similar sulphur-eating organisms at the foot of the Florida escarpment, where sulphide-rich hypersaline waters are seeping out at ambient temperatures onto the sea floor 3 km below the surface, show that our tube-worm friends are more plentiful than ever anticipated. The joy of all this for geologists is that we can learn much about subsurface hydrological processes while observing some of nature's true wonders. □

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Roger N. Anderson is at the Lamont-Doherty Geological Observatory, Columbia University, Palisades, New York 10964, USA.

Genetics

New aid to human gene mapping

from C. Thomas Caskey

ALTHOUGH the chromosomal localizations of the genes for Duchenne muscular dystrophy¹ and Huntington's disease² stand as powerful examples of the use of restriction fragment length polymorphisms (RFLP) to map human genes, considerable technical difficulties still limit the application of the technique. Help is at hand with the report on page 67 of this issue³ of a new family of DNA sequences, the characteristics of which are highly suited to RFLP linkage analysis.

Typical RFLP analysis has employed cloned functional gene sequences⁴, pseudogene sequences⁵ or 'anonymous' genomic probes², to detect what are presumed to be single base-change polymorphisms that generate or remove recognition sites for restriction endonucleases, the enzymes used to fragment DNA. Alternatively, these probes have been used to detect polymorphisms that are presumed to be due to DNA rearrangements⁶. With the exception of the last, these probes suffer from low heterozygosity because only two alleles are present in the population, one of which often occurs at a low frequency. As a result, no information can be obtained from some critical individuals in a pedigree or even entire pedigrees. Another problem is that genomic recombinants often contain highly repeated sequences that must be removed prior to analysis. Finally, closely linked polymorphic probes may not be available for a chromosomal region of particular interest.

Several of these difficulties should be obviated by the use of the DNA sequence family that is both tandemly repeated and dispersed in the human genome, and is described by Alec Jeffreys and his colleagues in this issue³. This family offers the opportunity to recognize multiple unlinked chromosomal loci and, with use of appropriate restriction endonucleases and specific probes, to identify variations in the number of copies of the tandem 'minisatellite' at each locus. The variation in copy number is seen as an RFLP by standard Southern blotting techniques and some loci are shown to be highly polymorphic for copy number of members of this repeat family (multiple alleles).

The minisatellite was first detected during characterization of the human myoglobin gene by Jeffreys and colleagues⁷ as an intron sequence that contains four tandem repeats of a 33 base-pair subunit and bears similarities to highly polymorphic sequences that are found near several other genes^{8,9}. A probe constructed as a concatamer of this repeat has now been used to identify numerous genomic lambda clones which contain sequences homologous to the repeat. Eight of the clones

were selected and further characterized. They differ in their base sequence, number of repeat units and, apparently, in their localization within the human genome (that is, they are not closely linked). All the repeat units, however, share a similar 'core' of 10–15 base pairs. Three of the eight repeats studied are highly polymorphic in copy number when hybridized under high stringency to DNA from various individuals. Pedigree analysis by Southern blotting shows that bands representing different copy numbers of repeats are inherited in a Mendelian fashion. Furthermore, the heterozygosity of these highly polymorphic repeats appears to be close to 100%. Thus, it is highly likely that these probes can yield information for analysing the pedigrees of families affected by a closely linked disease gene.

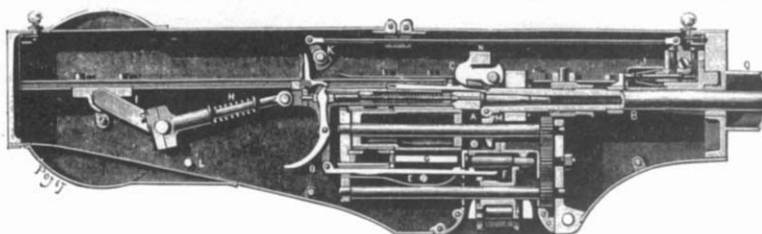
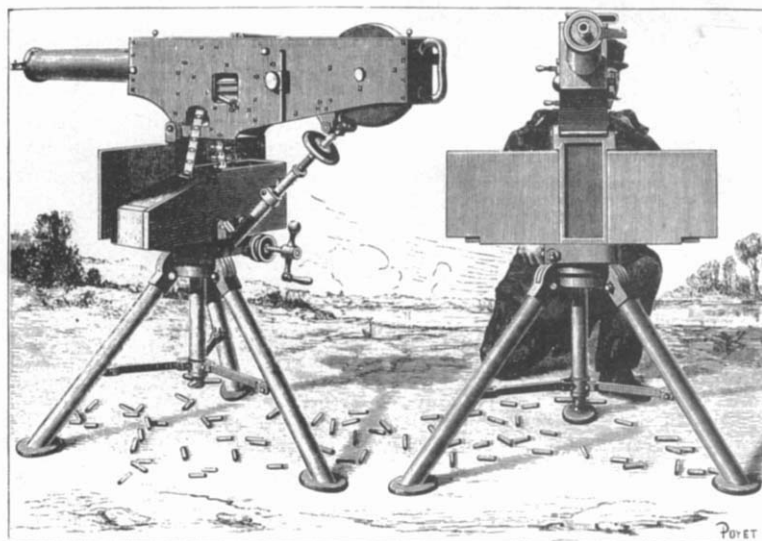
In order for these probes to be useful the chromosomal localization of the minisatellites will need to be determined. But it is clear that they provide an interesting and potentially significant improvement in the use of RFLP's for prenatal diagnosis, linkage studies, and gene mapping. Other polymorphic satellite DNA probes have

been reported^{10,11}, but they do not seem to have the same widespread potential in human gene mapping. Minisatellite sequence families offer the advantages of multiple chromosomal localization and greater heterozygosity at a given locus. Jeffreys *et al.* have described one, but their report holds out hope that other minisatellite families, which would make useful polymorphic probes available throughout the genome, await discovery.

Because the 'core' sequence of the repeated units resembles the generalized recombination signal of *Escherich coli*, a further use of these minisatellite sequences may be in the study of unequal crossing-over, the presumed mechanism for generating heterogeneity of copy number within a tandem repeat. □

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C. Thomas Caskey is in the Department of Medical Genetics, Baylor College of Medicine, Houston, Texas 77030, USA.

100 Years Ago The Maxim Gun From *Nature* 31 414, 5 March 1885.

MR. HIRAM STEVENS MAXIM, the well-known American engineer, has lately brought out a new form of a machine-gun, which is attracting a great deal of attention in military and naval circles. This gun is a completely new departure. It takes the cartridges out of the box in which they were originally packed, puts them into the barrel, fires them, and expels the empty cartridges, using, for this purpose, energy derived from the recoil of the barrel.

Lysosomes and prohormone activation

SIR — The interesting *News and Views* article by Pfeffer and Ullrich¹ refers to the unexpected relationship between the structure of the low-density lipoprotein and epidermal growth factor (EGF) receptors and that of the biosynthetic precursor for EGF itself. Pfeffer and Ullrich believe that a new clue to the puzzle is provided by the letter in the same issue of *Nature*² reporting the detection of unprocessed EGF precursor, but not EGF itself, in mouse kidney. The authors of this letter envisaged the possibility that the EGF precursor is a membrane protein and that it might function as a receptor in the cells of the distal tubules to regulate membrane transport events. This exciting new information, together with other clues to the puzzle, invites the re-evaluation of a suggestion I made a few years ago, prompted at that time by the related problem of the existence of prohormones^{3,4}.

In brief, it was proposed that polypeptide hormones have evolved from the constituent proteins of the secondary lysosome of simple unicellular organisms. A key element of this hypothesis was that the membrane of the secondary lysosome must contain transport processes. Hence the agents and products of lysosomal digestion would have direct access to, and the possibility of regulating, nutrient transport processes. Regulation of nutrition is a major feature of the action of many polypeptide hormones and growth factors. (As a result of this concept a glucose transport system was searched for and found in lysosomes⁵.) It was also proposed that the proteins which could act as polypeptide hormone precursors included membrane transport proteins and lysosomal digestive enzymes. "If some of the effectors arise by partial degradation of membrane proteins, then a structural relationship could exist between some effectors and receptors"³. A structural relationship between cobalamin binding proteins and their receptors has been recognized⁶.

Further informative structural relationships between a membrane protein, transport, nutrition and growth promoting activity, have emerged around transferrin and its receptor. The transferrin receptor shows weak homologies with the EGF precursor and chicken transferrin precursor⁷. Transferrin itself appears to be structurally and functionally related to an iron-binding cell surface glycoprotein which is present in most human melanomas, fetal intestine and, in trace amounts, normal adult tissues. This protein may translocate iron⁸. Transferrin has growth factor activity and stimulates the growth of some cells independently of its iron binding⁹. Finally the nucleotide sequence of a transforming gene suggests that it encodes a protein partially homologous to the amino terminus of transferrin, suggesting possible functional similarities¹⁰. It is possible that this smaller peptide is

growth-stimulating with a role similar to that proposed for platelet-derived growth factor in malignant transformation^{10,11}.

Pfeffer and Ullrich also mention that the precursor for a polypeptide homologous to EGF, transforming growth factor (TGF)- α , has a sequence suggestive of its being a membrane protein. It should perhaps be added in the present context that TGF- α and EGF show homology to the serine proteases urokinase and tissue-type plasminogen activator¹², and that EGF appears to be related to pancreatic secretory trypsin inhibitor¹³. The EGF precursor also appears to be structurally related to the serine protease factor X and more generally to the family of blood coagulation zymogens which includes prothrombin¹⁴. Thrombin itself has mitogenic activity and triggers a number of platelet reactions including secretion. These effects are not due to its protease activity but are considered to be hormone-like¹⁵. Other thrombin sequence homologies which have been described include those with angiotensin and the β -chain of luteinizing hormone¹⁶. It has recently been recognized that human γ -trace, a polypeptide having some homology with glucagon and corticotropin¹⁷, is an extremely potent cysteine-proteinase inhibitor¹⁸. The K_i for γ -trace inhibition of cathepsin L is less than 0.005 nM¹⁸. Thus a picture of close interrelations between some polypeptide hormones, proteases and their inhibitors emerges.

The sequence information which has emerged to date is consistent with the hypothesis that the lysosomes are involved in the activation of prohormones, suggesting that it may provide a useful framework for making and interpreting structural comparisons in this area.

C.N. HALES

Department of Clinical Biochemistry,
University of Cambridge,
Addenbrooke's Hospital,
Cambridge CB2 2QR, UK

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Agreement between optical and radiocarbon dating

SIR — D.J. Huntley *et al.*¹ recently described a new method for dating sediments. They compared their optically dated age of 62 ± 8 kyr for a sample with a radiocarbon dated age of 58.8 ± 0.3 kyr. In fact, the agreement is even better than Huntley *et al.* indicated, since the radiocarbon age used for comparison is based upon the wrong half-life for ^{14}C .

For well over twenty years, it has been internationally agreed to continue to use the Libby half-life value of 5,568 years for ^{14}C (ref. 2), although the best value was acknowledged to be a factor of 1.03 larger³. The procedure was justified by the desire to avoid the confusion which would arise should the many thousands of published dates require revision⁴. It should be noted that all radiocarbon dates are consistent among themselves but 3% lower than the true age.

As a result, the true (radiocarbon) age for Huntley *et al.*'s specimen would become 60.6 ± 0.3 kyr, which is in excellent agreement with their 62 kyr value.

NORMAN E. HOLDEN

National Nuclear Data Center,
Brookhaven National Laboratory,
Upton, New York 11973, USA

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Right-handed flagella in tumbling *Caulobacter*

SIR — In a pair of fascinating *News and Views* articles, Michael Spencer has recently discussed the remarkable case of bacteria that have right-handed helical flagella but are still able to reverse direction without tumbling, by simply changing the sense of rotation from clockwise to counterclockwise¹. There seems some uncertainty, however, about when such bacteria were discovered. Spencer presents data from the work of Alam and Oesterhelt on *Halobacterium* species². But the first bacteria to be discovered with such properties were in fact *Caulobacter crescentus* — they were studied by us in the University of Tokyo and the work was reported in an earlier edition of the same journal³.

SHIGEO KOYASU

Department of Cell Biology,
Tokyo Metropolitan Institute of
Medical Science,
3-18-22 Hongomage, Bunkyo-ku,
Tokyo 113, Japan

YASUO SHIRAKIHARA

Laboratory of Molecular Biology,
Medical Research Council Centre,
University Medical School,
Hills Road, Cambridge CB2 2QH, UK

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A tax not for imposition

On Budget day, 19 March, the British government may announce it is to impose value added tax on publications. The proposal might be economically rational but should be resisted.

FOR more than the past six months, British publishers and the British consumers of their products have been buzzing with anxiety at the proposal that there should be a tax on publications. The issue is simple. Should the government, in pursuit of its laudable ambition to shift the burden of taxation from income taxes to what is called indirect taxation (which applies to what people purchase), impose a kind of generalized expenditure tax on publications?

Such a move would be widely and fiercely scorned. On 19 March, when the budget for the coming financial year is known, it should be clear whether the British government is ready to go down yet another unpopular road.

That is why it is important not to exaggerate the case against a tax on publications. Value added tax (known as VAT) is in principle an economically rational and an equitable form of tax. The idea is that, in the manufacture of goods or the provision of services, each commercial entity will pay VAT on all purchases used in its business, collect VAT from all its customers, and hand over the difference between the output and the input tax to the government. At the end of each commercial chain stands the ultimate consumer, for whom VAT is a tax on expenditure. But because of the way in which the tax is structured, the burden is collected from all who have contributed to the production of something saleable in proportion to the value they have added to the final product. By now, VAT is a familiar part of the European scene, and even the basis on which the annual budget of the European Communities is calculated. As taxes go, VAT is a splendid principle. It would no doubt be more popular if it were more easily understood, and if its administration were not appallingly cumbersome.

In parochial Britain, for the past six months the popular slogan thrown at the proposal to levy VAT on publications is that such a move would be a "tax on knowledge", but that is not easily sustained. The most obvious difficulty is that there are many types of publication which can hardly be called educational or scholarly. Further, there are already many intellectual commodities in Britain and elsewhere in Western Europe which are taxed in exactly the same way. Those who buy tickets at theatres, opera houses and concert halls are already paying VAT. Who is to say that these institutions contribute nothing to the pool of knowledge of cultivated societies? That a performance of Beethoven's *Ninth Symphony* should be

taxed when *Pride and Prejudice* is exempt?

As things are, in Western Europe, something like 40 per cent of purchases of goods and services is subject to VAT, in most countries at a single percentage rate (which is, at present, 15 per cent in Britain). VAT is not levied on necessities of life such as food (except in restaurants) and rent, but unambiguously attaches to most manufactured goods, television sets for example. Publications are not taxed in this way, while commercial publishers are "zero-rated" in the sense that they neither pay tax on their inputs nor charge it on their products. Elsewhere in the European Community, in West Germany and, more recently, in Ireland, VAT applies.

So on what grounds should publications continue to be lumped in with food (but not drink) as necessities of life? The easy argument, that a tax that impedes the free flow of information within a democratic society is a threat to its well-being, is too riddled with inconsistency to be easily defended. One difficulty is that it has been readily, perhaps too readily, accepted that the commercial organizations willing to provide cable television services in Britain (and still waiting to see whether there is substance in the government's promise to "cable Britain") will be taxed by VAT. Another is that telephone calls, which are as powerful a means of conveying information as many publications, are also taxed, perhaps iniquitously. Compliantly to have accepted these taxes but to resist that now proposed for printed publications is inconsistent and, therefore, unwise.

The true case against the British government's flirtation with the proposal to levy VAT on printed publications is different and more pragmatic. There is a host of ways in which VAT would have disastrous consequences, one of which should be clear from the pages which follow where the virtues of a year's crop of textbooks are reviewed. A glance at the prices shown at the head of each review will be to most people a prompt for the question "how can students afford them?". So how will they get by if the cost is increased by another 15 per cent? Further depriving them of essential tools would throw doubt on the quality of education as such.

The case of learned journals is more complicated, and more shot through with potential inequity. The National Book Committee in Britain, an admitted lobbyist against the VAT proposal, has nevertheless produced some telling figures as part of its case. Surprisingly, it calculates that learned journals produced in Britain account for some 28 per cent of the titles in interna-

tional currency, but that the sales of these journals within the United Kingdom account for only 21 per cent of the total (reckoned as £187 million a year).

Even allowing for what may be very large errors in these estimates (based on replies to questionnaires), the figures suggest that the plan to levy VAT on publications would be a way of further and arbitrarily increasing costs in a modestly substantial export business where price-resistance is already strong. (Libraries everywhere are short of funds.)

The way in which the effects of VAT would discriminate between different kinds of customers and publishers would however be absurd. Commercial publishers (such as those of *Nature*) would at least be able to offset their input tax (paid out) against their output tax (collected from customers), but learned societies would have no such relief. Similarly, universities (already skimping unwisely on library acquisitions) would have no way of recovering the tax paid to publishers, but the libraries of commercial research laboratories, for example, would be able to deduct VAT paid on journals from that collected from the ultimate customers of their operations.

And while, in theory, VAT should be levied on imported publications, there is plainly no way in which this could be done on subscriptions serviced from abroad. (As Ireland has discovered to the cost of its own newspapers; VAT has been a boon to those who ship in British newspapers.) Yet a little discouragement of this kind could provoke a rapid emigration of many important journals that could be based anywhere in the world, and which are in Britain by historical accident. Is that what the British government wants to see occur?

But why not anticipate these untoward consequences of the imposition of VAT, and make arrangements that vulnerable groups of users, or publications, should be exempt? That seductive argument is the most dangerous of all, for it is a way of inviting the British government to discriminate between different kinds of publications. It amounts to censorship of a kind. Nobody, perhaps not even the government, wants that.

This is why a sensible decision about VAT on publications is bound to be a difficult decision. The government may like to think that the benefits of less direct taxation will put so much more money into people's pockets that the obvious economic consequences of the tax will be outweighed. In a more prosperous Britain, there might be something in that calculation. But as things are, VAT would further undermine the British academic enterprise, would begin a flight from Britain of many important institutions and would spread yet more intellectual impoverishment in a society that has long since lost its reputation for being widely read. Living with a little inconsistency is the better course. □

Matters of gravity

John D. Barrow

General Relativity and Relativistic Astrophysics. By Norbert Straumann.
Springer-Verlag: 1984. Pp.459. DM 112, \$42.

A First Course in General Relativity. By Bernard Schutz.
Cambridge University Press: 1985. Pp.376. Hbk £30, \$54.50; pbk £9.95, \$19.95.

General Relativity. By Robert M. Wald.
University of Chicago Press: 1984. Pp.491. Pbk \$34, £31.50.

EVER since the classic texts by Weyl and Eddington, written soon after the inception of general relativity, there has been a steady stream of high quality expositions of Einstein's route to his new theory of gravitation. Most have stayed close to the path first beaten by Einstein himself, which he described in the many editions of his own *The Meaning of Relativity*. Subsequent descriptions of general relativity have tended to focus upon polishing-up the standard route towards the field equations taken by Einstein, expounding the equivalence principle, covariance, tensor analysis, curvature and the field equations, before moving on to Schwarzschild's solution and the classical experimental tests of the theory. The situation is slightly reminiscent of the multitude of users' guides for the better known personal computers, each a minor revision of the manufacturer's manual.

Yet for all this duplication the didactic profile of general relativity has been dominated by a small number of influential books with very definite, but very different, points of view as to which aesthetic criteria should be emphasized in its formulation. While some authors regard the geometrical basis of the theory as the essential element in its formulation, others see this feature as an occlusion that inhibits its connection with the rest of physics. While one author may take a version of the principle of general covariance as its paramount guiding light, another may ignore it entirely or stress that it is empty of all physical content. This essential ambiguity in the initial motivation for entering into the mathematics along the lines followed by Einstein was the main reason for the proliferation of textbooks until the early 1970s. Since then, the motivation has changed: the development of more elegant and powerful coordinate-free formalisms has dominated the modern research literature in relativity and has begun to be promoted in new teaching texts. Further, there have been exciting new applications of general relativity to astrophysics and in the development of a unified approach to the natural forces. General relativity has finally ceased to be embarrassingly disjoint from the rest of physics, a haven only for group theorists and differential geometers.

These three books reflect the need for systematic expository texts that treat new theoretical and observational develop-

ments in modern mathematical language. Straumann's volume is the most formal in style, and has arisen from series of graduate lectures. It is divided into three parts which hang rather uneasily together.

The first part is a rigorous and detailed description of modern differential geometry and could be used as a course on this topic. Very clearly written, it takes the approach of the mathematician rather than that of the physicist; beginning with differentiable manifolds, it leads on to differential forms and the use of coordinate-free techniques for describing curvature. The second part then develops general relativity and contains detailed chapters on the behaviour of weak gravitational fields and the post-Newtonian approximation. Here, I found the exposition clear when the emphasis was on the mathematical formalism but rather weak on the link between physical principles and the mathematics. The last third of the book is much more physical and deals with relativistic astrophysics: neutron stars, the Kerr black hole and the laws of black hole mechanics, binary X-ray sources and, in the final chapter, the problems of accretion disks around black holes. All these astrophysical chapters are written at a high level but fall short of the standards set by Shapiro and Teukolsky's outstanding graduate textbook, *Black Holes, White Dwarfs, and Neutron Stars* (Wiley, 1983).

There are a small number of exercises scattered throughout the text but I did feel this to be an intended textbook that was written and printed in the somewhat forbidding style of a monograph.

Schutz is a well-known relativist who specializes in the study of gravitational radiation and equations of motion in general relativity. In this book he has put together a very carefully constructed course on general relativity which, unlike Straumann's, can be used by both undergraduates and graduates. Its great strength is the author's evident experience in teaching its content. The book is a goldmine of cleverly constructed problems and exercises (and solutions!) — there must be nearly 500 in total and a lot of careful planning has gone into their devising. This feature alone is sufficient to make this text far more appealing than Straumann's.

Schutz devotes the first 50 pages to recasting special relativity and vector analysis in modern formalism, before launching into tensors. Yet one wonders whether the level of detail here might not inhibit all but those able to chart a short-cut through this material from employing it as a core text, simply because of the limited length of undergraduate courses at British universities. But those seeking a basis for an advanced graduate course on the behaviour of gravitational radiation and black holes will find the author's evident mastery of the subject, and his ability to avoid mathematical formalism for its own sake, a strong attraction. The final chapter gives a brief but useful introduction to the homogeneous and isotropic cosmological models. Strongly recommended.

Wald's book is for a graduate course but, unlike its two competitors here, will be also extensively used by research workers because it is the first pedagogic treatment of so many advanced topics and techniques that form the research frontier of the subject. The author employs the so-called

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abstract index notation rather than traditional tensor notation. The formal treatment of manifolds, differential forms, continuous transformation groups, conformal transformations and the Hamiltonian formulation are all contained in extensive appendices that are designed to be integral parts of the text, displaced from the main body only on the grounds of continuity.

The first portion of the book covers the traditional ground from special relativity to Einstein's equations in a mathematical manner that I found was slightly lacking in its discussion of the physical motivation and content of the formalism; perhaps it is assumed that the reader has already followed a traditional introductory course? However, the outstanding part of this book is the treatment of a wide range of advanced topics in the second half of the text. Modern techniques for generating and classifying exact solutions to Einstein's equations, the causal structure of space-time and the use of techniques from differ-

ential topology to prove singularity theorems are all developed in detail. Each chapter is followed by a number of problems, although solutions are not supplied. The final series of chapters provides modern discussions of the initial value problem and Penrose's conformal treatment of infinity. These later chapters also focus upon topics close to the author's own research interests and there is a particularly nice introduction to developments in quantum gravity and the study of quantum fields in a classical curved space-time as well as detailed discussion of black hole mechanics and thermodynamics.

This is a much-needed book that will greatly stimulate the teaching of advanced graduate courses in modern gravitation physics. It will also provide an invaluable directory of modern techniques and results for gravitation theorists. □

John D. Barrow is a Lecturer in Astronomy at the University of Sussex.

Physical reasoning

Colin Upstill

Theoretical Concepts in Physics.

By M.S. Longair.

Cambridge University Press: 1984.

Pp.366. Hbk £25, \$49.50; pbk £8.95, \$14.95.

IT IS the exception rather than the rule for brilliant scientists to be the best teachers: Planck studied under Helmholtz and Kirchhoff, but confessed that "the lectures of these men netted me no perceptible gain". Most of us have at some time had the same experience, going full of expectation to a lecture by some professor of renown, only to find the lecture-room clock of more interest than his impenetrable phrases and even more impenetrable algebra.

Sadly, good textbooks are a rarity too. The texts whose content best matches our syllabuses are all too often hastily compiled pot-boilers, spin-offs from others' fumbling attempts at courses with similar titles to our own. It is of the greatest value in university teaching to possess a full and deep understanding of one's subject matter, yet to be aware of what it is like to be a student who does not. Professor Longair has this rare gift, and he has used it to good effect in writing this excellent book, subtitled *An Alternative View of Theoretical Reasoning in Physics for Final Year Undergraduates*. The subtitle hardly does the book justice; it is a complement rather than an alternative to most standard courses and standard texts — providing the cement of understanding to put between the blocks of lectures which go to build a physics course.

For most of the topics covered, Longair has learnt heavily on the best treatments

available elsewhere, and has often gone back to the original literature — for men such as Einstein, Maxwell and Rayleigh expressed lucidly ideas which subsequent generations of authors have muddled and obscured. It is no surprise to find that the book owes a great debt to Feynman's three-volume *Lectures on Physics* (Addison-Wesley, 1963–1965). But let me make it quite clear that Longair's book is much more than a collation of familiar material from diverse sources; throughout, it is stamped indelibly with the author's own deep insights and, equally important, his enthusiasm for his subject. This enthusiasm comes over vividly, and has resulted in a learned book with the rare property that it is compelling: like a well-crafted novel, it is hard to put down; at the end of each chapter, one turns the page eager to see what happens next. In my view, Longair has provided for final-year physics undergraduates the sort of essential background Feynman gives them in their first and second years. No facet of the reality of the practice of physics is omitted, from the importance of experimental observations for theory (via Kepler's use of Tycho Brahe's observations and especially his error analysis, which was so crucial to the discovery that the orbits of the planets must be elliptical rather than circular), through the use of models (such as Maxwell's mechanistic models for electromagnetic phenomena), to the use of computers (to simulate the synthesis of light elements in the hot big bang).

In his epilogue, Longair admits that in preparing the book he learned a great deal which he wished he had known years ago. I feel the same after reading it. □

Colin Upstill is a B.P. Venture Research Fellow at the H.H. Wills Physics Laboratory, University of Bristol.

Round the mechanics course

J.W. Humberston

Classical Mechanics*.

By B.P. Cowan.

Routledge & Kegan Paul: 1984. Pp.111.

Pbk £3.95, \$9.95.

CLASSICAL mechanics continues to be regarded as an essential component of a modern degree course in physics, and this book attempts to cover all the material required by a student taking a first course in the subject. It is a rather bold claim to make of a book containing only 111 pages, but the choice of topics is generally satisfactory. There are chapters on Newton's laws, energy and momentum, dynamics of a single particle, many-particle systems and rigid bodies, and non-inertial frames. The final chapter gives a very clear introduction to Lagrangian and Hamiltonian mechanics, ending with a brief section on Poisson brackets and their relationship to commutators in quantum mechanics. Worked examples are distributed throughout the book and a short set of problems (with solutions) is also included.

Because the book is so small, there are, inevitably, omissions. For me, the most serious of these relates to moments of inertia. After a brief account of the inertia tensor there is merely the statement that the diagonal elements are the moments of inertia. No mention is made of the motion of a rigid body about a fixed axis (unless it be a principal axis) nor of the theorems of parallel and perpendicular axes. There is not even a worked example of the calculation of a moment of inertia.

Also, in the discussion of motion under an inverse square law of force, insufficient detail is given of the properties of conic sections. It is assumed, I would have thought unreasonably, that the reader is already familiar with them. A bibliography of more extensive works in mechanics would have been some compensation for these and other omissions but, unfortunately, one is not provided.

The book is written in a clear and concise style and, despite its shortcomings, should generally be well received by its intended readership. □

J.W. Humberston is a Lecturer in the Department of Physics and Astronomy at University College London.

*A volume in Routledge & Kegan Paul's *Student Physics* series. Other titles to appear during 1984 were *Quantum Mechanics* (by Paul Davies), *Relativity Physics* (Roy Turner) and *Electricity and Magnetism* (Roland Dobbs). All are short paperbacks costing £3.95, \$9.95. Forthcoming later this year are *Electromagnetic Waves* (Roland Dobbs) and *Liquids and Solids* (Michael Sprackling).

Good companions

Robert Cannon Smith

Astrophysics. Vol. I Stars. Vol. II Interstellar Matter and Galaxies.
By Richard Bowers and Terry Deeming.
Jones and Bartlett/Wadsworth: 1984.
Vol. I pp.344, Vol. II pp.244. \$37.50,
£17.95 each volume.

THE main teaching load for astronomers in the United States is the large first-year class of liberal arts students and so there are many textbooks jostling for a place in that market. In British universities it is more common to teach astronomy only to science specialists, usually in the context of physics courses. Good books at that level are rarer and so it is a pleasure to find a new two-volume work that caters for the advanced undergraduate or beginning graduate student.

In 588 pages the books attempt to cover the whole of modern astrophysics, excluding the Solar System. The task is made easier by the assumption that the reader already has an elementary knowledge of astronomy, presumably from one of the myriad first-year descriptive texts, but nonetheless the authors succeed remarkably well in providing a comprehensive coverage at a useful depth. They are not content with order-of-magnitude arguments — though these are well-used where

appropriate — but give proper mathematical derivations while managing to avoid the generality of treatment that only serves to confuse the new student.

This is not a research monograph and so is not completely up to date. For example, the discussion of supernova models mentions radioactive decay as an explanation for the exponential tail in the light curve of a Type I supernova but attributes the decay to Cf^{254} rather than to the more likely Co^{56} . In the more general discussion of stellar evolution, there is little emphasis on mass loss, except through mass transfer in binary systems. However, the volume on stars is more complete than its companion, in which there is little hint of the modern picture of the violent interstellar medium, and almost no discussion of interstellar molecules. The exciting subject of active galactic nuclei and quasars gets essentially no mention at all. These and other omissions make the books more suitable

for undergraduates than for graduates, though the latter will learn useful things about longer-established branches of astrophysics. Even in these branches there are some surprising gaps, however, such as the absence of any detailed account of how the rotation curve near the Sun is found. As a whole the second volume is not as good as the first, being less complete and less balanced. It also stops rather abruptly, with no attempt to draw ideas together.

Still, I liked these books. They are readable and well-illustrated, full of short but useful problems and include some helpful suggestions for further reading. Without producing something twice the length it is doubtful if a much better job could have been done, and I shall certainly be including them on reading lists for my own students. □

Robert Cannon Smith is a Lecturer in Astronomy at the University of Sussex.

Out of this world

David W. Hughes

The Universe of Galaxies: Readings from Scientific American.

Compiled by Paul W. Hodge.
W.H. Freeman: 1984. Pp.113. Hbk \$20.95, £23.95; pbk \$10.95, £12.50.

A Workbook for Astronomy.

By Jerry Waxman.
Cambridge University Press: 1985. Pp.356. Comb bound £15, \$19.95.

Planets and Their Atmospheres: Origin and Evolution.

By John S. Lewis and Ronald G. Prinn.
Academic: 1984. Pp.470. Hbk \$59, £49.50; pbk \$29.50, £25.

The Solar System.

By Barrie William Jones.
Pergamon: 1984. Pp.336. Hbk £24.25, \$40; pbk £11.75, \$19.50.

ASTRONOMERS at the chalk face of tertiary education have a lot to be grateful for, not the least being the hard work of authors and publishers. Astronomy has popular appeal, and so attracts the attention of glossy scientific journalism; it has a strong practical edge and many new ideas for laboratory experiments have been laboriously written up into book form; and it is fast moving, an attribute that results in a stream of updated textbooks.

At the forefront of the popularization of astronomy are the monthly astronomical reviews that appear in *Scientific American*, noteworthy for their clarity of exposition — both in text and in figures — and the erudition of their authors. Periodically the publishers gather together articles on a specific topic and issue them in a large-format paperback. *The Universe of Galaxies* is one such collection. It starts with a paper by Bok on our own galaxy and moves out, encompassing the Andromeda

galaxy, dark matter in spiral galaxies, the evolution of disk galaxies, tides between galaxies, active galaxies, cosmic jets, superclusters, voids and quasars. The important word in the title of the book is *Readings*. It has never been easy getting students to read and *Scientific American* must be praised for coaxing many and enlightening most.

Practical work is crucial to astronomy — observations must be made, spectra analysed, graphs plotted, errors calculated and results mused over. But how do we introduce this aspect of the subject to the throngs in arts faculties that take a science course in astronomy at American universities. These students dive into astronomical texts by the likes of Abell, Pasachoff and Hartmann and read, somewhat grudgingly, enough of them to pass the course. They must be encouraged to wander into the laboratory planetarium and observatory. Waxman's book is a valuable start, providing a complete set of practical activities to supplement the factual meal to be gleaned from the textbooks. Suggested activities are divided into three time categories, short ones which can supplement a lecture, longer ones suitable for a three-hour laboratory class and others concentrating on outside observations which can be carried out individually and can extend over many weeks.

A Workbook for Astronomy contains a multitude of bright ideas and, what is more important, the wherewithal for carrying out the experiments — finder charts for binaries, comparison spectra, lunar charts, reproductions of crater fields, star charts and photographs of clusters of galaxies. Students are introduced to a wealth of topics including stellar evolution, distance measuring, planetary orbits, the curvature of space, the age of clusters and classification of galaxies. They learn by doing. I found the book a source of many good ideas but for British science teaching the standard is rather elementary. Also,

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while I can see very little advantage to the student in having the book held together by a plastic ringbinder, the advantage to the publisher is obviously enormous. After one year of use the book falls to pieces and the owner cannot recoup some of his outlay by selling it to those who follow. The quest to reduce the second-hand value is also helped by including "data sheets" and graph paper (with marked axes!) in the text. This surely spoonfeeds students too much, for one of the objectives of laboratory exercises is to introduce them to the craft of tabulating results and planning graphs.

The other two books reviewed here both deal with our Solar System. Lewis and Prinn have provided, in *Planets and Their Atmospheres*, the ideal starter pack. It is not for the faint hearted, however, being aimed accurately at the final-year student, postgraduate and research worker. To this end the authors have assembled an impressive 70 pages of references and a detailed, itemized index which has at most five page numbers for each topic. The book is also timely. Spacecraft have provided an enormous bank of data about the present atmospheres of the terrestrial planets Venus, Earth and Mars. Observations of Jupiter, Saturn, Uranus, Neptune, Titan and Io have also helped us formulate models for the structure, composition and chemistry of their gaseous mantles. Detailed comparative studies have allowed us to move away from the present state to a discussion of origins and evolution.

Why do I call it a "starter pack"? Well, when considering a new topic, all researchers and students need to read a few sections of a good book to familiarize themselves with the relevant background before turning to shelves of journals in the library. There are too few good books of this type around. We must be grateful to Lewis and Prinn for providing another.

Jones's *The Solar System* is a non-mathematical introduction to our planets. I found it rather difficult to visualize the audience it is intended for. We are given a contemporary picture of the planets, with many references to the new data obtained from spacecraft, but the view has been somewhat drained of its excitement. Everything is mentioned, but only briefly and sketchily, and the author has thus given himself little room to point out the problems and mysteries. He has also taken the rather old-fashioned approach of compartmentalizing each planet into a separate chapter, which militates against fruitful comparisons. And now that we are so used to pages of colour images of planets, the reversion to black-and-white produces a sense of disappointment.

There are many general books presently available which deal with the Solar System. Unfortunately, most of them are better than this one. □

David W. Hughes is Senior Lecturer in Astronomy and Physics at the University of Sheffield.

From eye to CCD

Michael Rowan-Robinson

Astrophysical Techniques.

By C.R. Kitchin.

Adam Hilger: 1984. Pp.438. Hbk £40, \$65; pbk £15, \$25.

IN THE 50 years since Jansky first detected the Milky Way at radio wavelengths there has been a phenomenal explosion in techniques of astronomical observation. The new wavelengths — radio, X-ray, ultraviolet, infrared, microwave, gamma-ray — and the other types of astronomical information reaching the Earth, such as neutrinos, cosmic rays and gravitational radiation, have each required new detection techniques, new types of telescope and new observing platforms, ranging from caverns deep under the Earth to satellite-borne telescopes. Optical astronomy too is being revitalized by new techniques, for example the CCD (charge-coupling device) array and the fibre-optics spectroscopy.

Many university and polytechnic departments of physics or astronomy give courses on astronomical observing techniques and instrumentation. Not all of these bear much relation to what is actually happening in astronomy today, and there is a great need for authoritative and accessible books

on the subject. Dr Kitchin has set out to provide an account suitable for the science-based undergraduate, though he hopes that the amateur astronomer will find it useful and also points out that increasing specialization in the subject means that most professional astronomers would also benefit from such a book.

Dr Kitchin concentrates on techniques of detection and imaging, but extends this to include the basic optics used in modern telescopes. There is little discussion of the atmospheric and other background limitations which pose such a challenge to observational astronomy and which drive so many of the technical developments. The book is crammed with useful detail, though. It is strongest on the techniques of optical observation: the coverage of the new astronomies such as radio and X-ray is fairly skeletal. Microwave and submillimetre developments are not mentioned. The physical basis of the techniques is briefly discussed, but a fair knowledge of atomic physics and optics is implicitly assumed.

This will be a useful addition to the reading list for a more advanced course on astronomical techniques. But the amateur astronomer may find it a bit heavy going.

Michael Rowan-Robinson is Reader in Astronomy at Queen Mary College, University of London.

Inside the planets

D.C. Tozer

Physics of Planetary Interiors.

By G.H.A. Cole.

Adam Hilger: 1984. Pp.208. Hbk £22, \$39; pbk £9.95, \$16.

THERE will always be a need for books that try to put the enormous complexity of the known planetary structure and behaviour into a readily comprehended form — books that show a student how much can be explained by a relatively straightforward application of generally accepted physical principles. Professor Cole's book is directed at undergraduates and his choice of subject and approach reflect his background in applied mathematics. For example, much — in my view, too much — attention is given in this introductory text to the gravitational fields and shapes of rotating massive bodies in hydrostatic equilibrium (though who would now deny that a world of meaning and whole fields of research have been inspired by the very small but definite departures of the actual geopotential surfaces from such predicted shapes?). A student needs much more guidance than he is given here for pursuing calculations that tacitly imply a very static view of planetary evolution.

Characteristically, an attempt is made to define the realm of planetary science as that

of objects in which self-gravitation is strong enough to induce an axial symmetry of their external shape, but not so strong as to make the atoms "incapable of maintaining the equilibrium which defines a planetary body". Such efforts at classification appeal to the tidy mind, but are likely to prove very confusing to the able and inquisitive student. "Minor planet" is a well-established usage for objects whose light curve suggests a highly irregular shape, and if the quotation I have taken is intended to mean no pressure ionization of atoms, what is one to call an object such as Jupiter, in which a substantial mass fraction is believed to be a pressure-induced metallic phase? Further clarification is not helped by the erroneous comment that free electrons are non-relativistic in planets but fully relativistic in white dwarf stars.

Unfortunately, this kind of ambiguous or poorly reasoned remark is all too common — I noticed serious conceptual errors and typographical mistakes that make the sections on planetary heat transfer and magnetism positively misleading. If there is no treatment of seismology, a chapter devoted to a hydrostatic theory of the structure of icy major planet satellites is an original feature. Nevertheless, I would not recommend this book to students until several corrections have been made. □

D.C. Tozer is a Reader in Theoretical Geophysics at the University of Newcastle upon Tyne.

Eumorphous amorphs

Robert W. Cahn

Physics of Amorphous Materials.

By S.R. Elliott.

Longman: 1984. Pp.386. £25, \$60.

ONE of the joys of contemplating amorphous materials is their affinity with paradox. *Amorphous*, etymologically, signifies *shapeless, without form*; yet the book under review has an 80-page chapter on structure. One of the key theorems general to all amorphous solids is the Kauzmann Paradox, which tells us that for all glasses (a concept not quite coterminous with amorphous solid) there is a temperature above 0K at which the entropy must decrease below that of a crystal of the same composition; Dr Elliott offers a careful discussion of the implications of this paradox, which belongs to the category of impossible things that Lewis Carroll's White Queen liked to believe before breakfast.

Again, Dr Elliott repeatedly insists that an amorphous material has no reciprocal lattice, and this poses enjoyable obstacles to theorists who seek to understand, in particular, electron transport in these materials. But even this "reciprocity failure" may not be a universal attribute of amorphous materials, as witness the publication, a few weeks ago, by Shechtman and others, of evidence of a non-periodic metallic phase (a quasicrystal), neither glass nor crystal, which yet gives a sharp diffraction pattern... of fivefold symmetry!

There is a plethora of textbooks and monographs on glasses, but until recently the word has tacitly connoted oxide glasses. A leading exemplar of this large category is *Glass Science* by R.H. Doremus, published in 1973, which is entirely about oxide glasses, an excellent book, subject to that limitation. J. Zarzycki's *Les Verres et l'Etat Vitreux* (1982) takes this classic approach to glass science as far as it will go. Lately, a flood of literature, but no textbooks, about metallic glasses has appeared. Other textbooks again, notably Mott and Davis's celebrated *Electronic Processes in Non-Crystalline Materials*, have covered the theoretical physics of conduction in chalcogenide glasses.

Elliott approaches the field in a new way. He treats amorphous materials quite generally in terms of preparation strategies, ease of glass formation, the glass transition and its statistical mechanics and thermodynamics, and, in particular, structure. That chapter has the clearest exposition of diffuse diffraction from glasses and of EXAFS that I have seen. Defects are also discussed; of course, a genuinely formless material could not possibly have defects, since the existence of defects implies the existence of a non-defective structure! The author pursues a resolutely quantitative

approach but combines it with frequent pauses to see where he has got to in terms of a physical model. The reader is thus helped to keep track of the argument. Problems at the end of each chapter, linked always to the relevant mathematics, will help the conscientious reader to cement his understanding.

The heart of the book is a more specialized theoretical treatment of spectroscopy and electron transport, mostly with reference to semiconducting (chalcogenide) glasses but also to metallic glasses. A number of difficult topics are painstakingly set out; I have never seen such a clear discussion of the difficult concept of two-level

systems. However, as the author himself specifies, the reader must be competent in solid-state physics somewhat beyond the level of Kittel's *Introduction to Solid State Physics*, and this distinctly narrows the readership.

This fine book has at present no effective rival. Although — inevitably — it barely touches on some aspects of some types of amorphous solid, it is yet an excellent basis for an advanced course on the amorphous state for physicists. □

Robert W. Cahn is currently Visiting Research Fellow at the General Electric R&D Center, Schenectady, New York.

On the ground

C. Kidson

Geomorphology.

By Richard J. Chorley, Stanley A. Schumm and David E. Sugden.

Methuen: 1984. Pp.605. £17.95, \$30.

The Nature of the Environment: An Advanced Physical Geography.

By Andrew Goudie.

Basil Blackwell: 1984. Pp.331.

Hbk £22.50, \$39.95; pbk £7.50, \$14.95.

Environmental Systems: An Introductory Text.

By I.D. White, D.N. Mottershead and S.J. Harrison.

George Allen & Unwin: 1984. Pp.495.

Hbk £30, \$50; pbk £11.95, \$19.95.

MORE than 80 years ago, long before the current environmental movement began, geography was described as "the study of man in his environment". However physical geography, environment and geomorphology, as covered by these three volumes, have differing meanings. There is, nevertheless, sufficient overlap to make it useful to look at them together.

In the narrower field of geomorphology, concerned with the geometry of the surface features of the Earth, the new work by Chorley, Schumm and Sugden must be hailed as a masterly review of the development of the science and of its present status and subject matter. It will rank with the classical presentations of Lobeck (1939) and Von Engel (1948). Its publication may, indeed, come to be regarded as marking almost as significant a stage in the evolution of the subject as the major works of Davis (1909), Penck (1924) and King (1967). Time will be needed to test this view, which some may regard as premature and perhaps extravagant.

The great merits of the book are its balanced presentation of the different ways of looking at the development of landforms and the integration of the historical and functional approaches. Part 1 represents the clearest, most concise and penetrating introduction to the subject which it has been my pleasure to read.

While the whole work is clearly aimed at advanced students and professional geomorphologists, this section can be recommended even to first-year students. This is so even though they may not fully comprehend the whole of it and despite the fact that it does less than justice to the studies of denudation chronology, largely carried out by students of that greatest of English Davisian disciples, S.W. Wooldridge. Part 3 is a comprehensive review of contemporary Earth surface processes which brings the advanced student to the very frontiers of research. The remaining sections on geological (Part 2) and climatic geomorphology (Part 4) are penetrating syntheses of these areas of the subject.

The book as a whole is a balanced statement of the present position in geomorphology. The specialist interests of the authors have been combined in a most successful unified approach, and while it is sometimes possible to detect the authorship of particular sections only those very familiar with their individual work will be able to do so.

It is probably unfair to compare this outstanding volume with the new study of the environment by Professor Goudie, which is sub-titled *An Advanced Physical Geography*. This book covers not only geomorphology but includes also climatic and ecological sections and a consideration of man's impact on the environment. Nevertheless, by far the greater part of the subject matter is the same as in Chorley *et al.* In addition, the publishers claim that Goudie's book "is informed by the latest advances in theory and research" and that it "will surely become the standard work". For these reasons alone comparison becomes inevitable.

It has to be said at once that *The Nature of the Environment* suffers in the comparison. It is written in a discursive and descriptive style, with frequent statements of the obvious such as "The great bulk of the world's water is in the oceans" and "During glacials the ice caps of the world were greatly expanded" which one might expect to find in a first year essay. One example serves to show that the publisher's claim for a research basis is somewhat

overdrawn. The short and inadequate section on sea level changes shows no acquaintance with the work, between 1974 and 1982, of the International Geologic Correlation Project No.61, on sea level change in the last 15,000 years. Similarly the claim that it will become the standard work looks somewhat exaggerated in the light of a reference list confined solely to textbooks. In short, the book has some merit as an introduction to the subject but is not for advanced students and research workers.

The last of this trilogy, *Environmental Systems*, is a modest approach to the very

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Subject for the geomorphologist — the Grand Canyon, drawn c. 1880 by William H. Holmes.

broad subject of "the environment". As an introductory text, which it claims to be, it has much to offer. Unlike Chorley *et al.* the authors do not set out to embrace, or even to reconcile, all approaches to their subject matter but concentrate firmly on the single methodology of their title. In this the book is very largely successful. A careful factual exposition is supported by frequent references to source material, by detailed suggestions for further reading and by a bibliography of 350 journal references. It is a little disappointing that Part C does not include any treatment of coastal environments — the book's aim to promote "an understanding of Man's terrestrial environment" surely cannot be entirely achieved if it ignores a part of it where such a large number of people live and work. Despite this shortcoming this is a successful book and one which can be recommended to the readership at which it aims.

The strength of interest in "the environment" is shown by the steady flow of publications in this general field at all levels. Occasionally an outstanding book in a specialized part of the subject appears and tends to overshadow other worthwhile contributions. *Geomorphology* by Chorley, Schumm and Sugden is such a work. Still, the other volumes reviewed here also have their strengths and will be of value to somewhat different readerships. □

C. Kidson is Emeritus Professor of Physical Geography at the University College of Wales, Aberystwyth.

Devonian review

Bruce Sellwood

Aspects of a Stratigraphic System: The Devonian.

By D.L. Dineley.

Macmillan, London: 1984. Pp.223. Hbk £20; pbk £9.95.

THE Devonian Period spans some 48 million years (408–360 Myr) and was originally established, in Devon, as the Devonian System by the entrepreneurial stratigraphic approach of Sedgwick and Murchison in 1839. David Dineley, who has devoted many years to research on the Devonian and its fish faunas, has written this book to illustrate, he says, the ways in which the different disciplines within geology help our understanding of how things were 360 million years ago. He claims that it is aimed at the "interested" but "not very advanced student of historical geology".

The book doesn't really provide a source on regional Devonian geology (though in fairness one should say that it doesn't set out to do so); thus it cannot be used in the same way as Arkell's masterly descriptive account, *Jurassic Geology of the World* (Oliver and Boyd, 1956). Neither does it provide an ideas approach to a system comparable with that of Hallam's *Jurassic Environments* (Cambridge University Press, 1975). Instead it falls somewhere in between.

The introduction attempts to justify use of the Devonian as a vehicle for the book "because it is there", an argument that also recurs in subsequent chapters on the materials of stratigraphy, and stratigraphy and the world tectonic model. In the latter, plate tectonic models are extensively adopt-

ed from the literature, although being considered "crude means of solving problems in crustal history". The next chapter ("Time in Question") shows how stratigraphic correlation is achieved, while Chapter 5 deals with biostratigraphy and reviews the various groups of organisms found in Devonian rocks and their use in correlation.

The following four chapters deal with Devonian facies: Old Red Sandstone; paralic facies (including some carbonates); carbonates and evaporites; and deep-water suites. Each begins with a generalized global review before giving brief accounts of individual areas (Arctic Canada, western Europe, the Russian Platform, Altai-Sayan, east Asia and so on). Many maps are included (though only a single isopachyte map occurs in the whole book), but relatively few stratigraphic sections. This is a pity in view of the fact that, after correlation, successful stratigraphic interpretation rests on the details of sequence evaluation.

Finally, Dineley considers Devonian geography, particularly covering recent work on possible continental dispositions, facies and the oceanographic implications. Climatic changes are reviewed and notions of meteorite impact speculated upon.

I'm unsure about the readership for this book. It cannot really be for "interested" students, because they will get lost in the sections on biostratigraphy; and it won't be for the specialist Devonian workers because this is a broad scale review. Still, it should be taken up by university libraries where it will be a useful student text for the few sessions normally spent on Devonian geology. □

Bruce Sellwood is a Lecturer in Geology at the University of Reading.

Earth through time

A. Hallam

The History of the Earth's Crust.

By Don L. Eicher, A. Lee McAlester and Marcia L. Rottman.

Prentice-Hall: 1984. Pp.197. Hbk \$25.60, £23.30; pbk \$22.90, £20.85.

The Evolving Continents, 2nd Edn.

By Brian F. Windley.

Wiley: 1984. Pp.399. Hbk £25, \$24.95; pbk £9.95, \$21.50.

IN THE current enthusiasm to understand more about geological processes in such diverse fields as sedimentation, rock deformation and volcanism, increasing use is made of mathematical modelling and modern laboratory techniques. This analytical approach remains somewhat sterile, however, if it fails to illuminate Earth history, which remains at the core of academic geology. Because the subject is so complex and involves synthesis of a

diversity of rapidly growing research fields, satisfactory undergraduate textbooks are few and far between. As in other spheres of science the premium in research is on increased specialization and this inevitably influences what is taught in university courses.

The advent of the new book by Eicher, McAlester and Rottman is to be welcomed because, within its limitations, it copes very well with a difficult task. The principal limitation is one of brevity; the book forms part of a Prentice-Hall Earth Science series which is restricted to short, small-format books. The text is clearly written in plain, straightforward English and there are plenty of good illustrations. After an initial outline of stratigraphic principles and geochronology the authors deal with the Earth as a planet in a way more customary in physical geology texts, before devoting successive chapters to the Precambrian, Palaeozoic, Mesozoic and Cenozoic.

The writing is thoroughly up to date, whether dealing with subjects as different as Precambrian life and the cause of mass

extinctions, suspect terrains or orbital cycles as a cause of ice ages. The principal shortcoming is that the book is written at a very elementary level and has a minimal bibliography. There is little critical evaluation or in-depth treatment of specific subjects, and detailed examples are taken almost exclusively from the United States. This is essentially an Earth history digest aimed at the large American freshman and sophomore market; in consequence, it is unlikely to be adopted widely for geology honours courses in British universities, where world history is normally dealt with at a comparatively advanced level after a thorough grounding in stratigraphic principles has been undertaken with reference to local examples.

For the past eight years the most suitable textbook for senior undergraduates has been Windley's *The Evolving Continents*, in which a pioneering attempt was made at an overview of continental evolution from the Archaean onward, within a conceptual framework of plate tectonics. Windley's book has been a well warranted success and the second edition is timely.

So rapid have been the advances of recent years that substantial revision has proved necessary, though the basic outline remains unchanged. The new Harland timescale is adopted uncritically in a new opening chapter. It is perhaps as well that Windley did not wait a further couple of years before revision, because he would then have had three other new timescales to contend with, all differing in some important respects. Besides major changes in the Precambrian chapters there are new sections for the Phanerozoic on a host of subjects. These include, among many others, phosphorites in relation to oceanic upwelling, mass extinctions, the Iapetus Ocean, the elevation and climate of Pangaea, oceanic mineralization, Pacific island arcs, suspect terrains and the structure and evolution of the Himalayas. The bibliography remains at 30 pages but the contents are much changed.

As in the first edition, Windley is at his most authoritative when dealing with tectonics, especially the Archaean and Proterozoic, though he is not sufficiently up to date to record that much of eastern Asia is now thought to consist of isolated continental fragments brought together since the late Palaeozoic. On subjects such as palaeoenvironments, or those dealing with events in the biosphere, he is less comprehensive and reliable. The big advances recently in the understanding of sedimentary basin formation and continental margin subsidence are largely ignored, and Phanerozoic sea-level changes receive only a scant treatment. It would, however, need a superman to produce an advanced textbook of Earth history that was equally strong in all fields. □

A. Hallam is Lapworth Professor of Geology at the University of Birmingham.

Student pollution

Peter S. Liss

Fundamentals of Air Pollution, 2nd Edn.

By Arthur C. Stern *et al.*

Academic: 1984. Pp. 530. \$39.50, £28.

Pollution of our Atmosphere.

By B. Henderson-Sellers.

Adam Hilger: 1984. Pp. 210. Hbk £30, \$49; pbk £13.95, \$23.

AIR POLLUTION is a fast-moving topic which has attracted much public attention in recent years. Developments in it have often been haphazard since, instead of a steady flow of funding for research, there has been a tendency to throw large amounts of money at particular problems, for example acid rain, and halocarbons and their possible impact on stratospheric ozone. With this sort of patchy temporal progress it might be thought that there would be widespread disagreement as to what should be included in the corpus of knowledge constituting the subject. However, to judge from these two books there is apparently considerable unanimity as to what courses in air pollution in higher education should contain. Basically both of them deal with sources, effects, measurement, monitoring, meteorology, and regulatory and engineering control of air pollution. But there the similarity ends.

The book by Stern *et al.* is a new edition of a publication of the same title which appeared 11 years ago. Two of the original four authors remain and two new ones have been recruited. The style and level of the new edition are much as previously, but the text has not merely been revised and

considerable parts of it are completely new. Even so, there is a less than modern air about it, a possible reason for which will be discussed shortly. Students will find the book packed with information and it will serve them not only while they are at college but also in any subsequent career concerned with atmospheric science.

Henderson-Sellers's book is definitely a new production but again it doesn't strike me as really being at the forefront of knowledge. It is readable and a real attempt is made to explain how physical processes operate in the atmosphere, rather than merely stating that they do, as is done in less didactic treatments. The book is quite short and students should find it a good read, though I'm not sure it will be as useful to them after graduation as will Stern *et al.*

My principal grouse about both books is their lack of treatment of atmospheric chemistry. Both attempt to deal with chemical aspects of the subject but the approach is peripheral rather than central. Of the two, Stern *et al.* does a better job in this regard but even here the chemistry has insufficient weight. Because of this, important topics such as removal of air pollutants by natural cleansing processes, hydroxyl chemistry, and even acid rain receive only scant attention. It is the underplaying of chemical aspects which I believe gives both books a somewhat dated air. Arguably the biggest growth area in air pollution research in the past decade has been in atmospheric chemistry. Books in which insufficient attention is paid to this will inevitably look old before their time. □

Peter S. Liss is Reader in the School of Environmental Sciences at the University of East Anglia.

Archaeology anew

Colin Renfrew

Prehistoric Europe.

By Timothy Champion, Clive Gamble, Stephen Shennan and Alasdair Whittle.

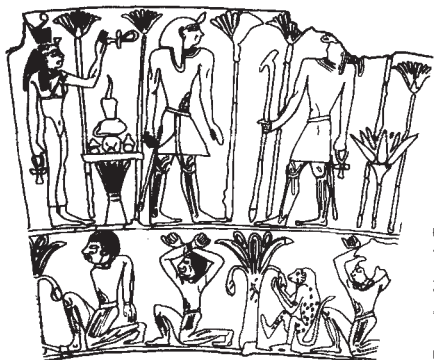
Academic: 1984. Pp. 359. Hbk \$45, £28.50; pbk \$23.50, £14.50.

WHEN a discipline has recently undergone a profound, perhaps revolutionary transformation, the first textbook to follow that upheaval is of more than usual significance. The study of European prehistory has indeed undergone such a transformation over the past 15 years, with the widespread acceptance of a chronological system very different from that which prevailed earlier, and with the development of interests and forms of explanation which were not much in evidence then. This ambitious volume offers the first reasonably comprehensive overall coverage of the resulting subject, from the palaeolithic period to the iron age.

Three qualities separate the prehistoric archaeology of today from the traditional version, seen in a series of standard works from V. Gordon Childe's *Dawn of European Civilisation* (first edition 1925) to Stuart Piggott's *Ancient Europe* (1965). The first is the happy lack of preoccupation with chronology: radiocarbon dating has now successfully solved many of the outstanding chronological problems. So that while the early works of the new radiocarbon era were backed up by bulky appendices of date lists, much needed at the time, these are absent here. A second feature is the refreshing absence of automatic diffusionist assumptions: no longer is it taken as self-evident (whether by explicit axiom or by unstated convention) that most changes in the prehistoric record came about as the result of contacts with the civilized lands of the Near East by a process of the "diffusion" of culture. And thirdly, the processual approach now predominates: that is to say the emphasis is on the explanation of change through an analysis of economic conditions and social structures. The discussions of change in

this volume are lively and interesting, and in some cases new and original as well.

The book is the collective work of four authors: justifiably so, for there is no one today who could cover the subject single-handed with the same authority. Indeed perhaps there never was, for the earlier textbooks in general did not venture very far back into the palaeolithic period. Inevitably, however, in the necessary work of compression some detail is lost. We no longer see the comprehensive summaries of pottery styles and of the artefact forms



From Prehistoric Europe

Pictures of the past — decoration on a vase dating from the late eighth or seventh century BC found at Tarquinii in Etruria.

which occupied so large a proportion of many earlier accounts. Instead the themes treated are sometimes at a further remove from the raw data. In consequence this book will perhaps be more vulnerable to change as problems come to be solved and new questions arise. But, in a sense, the better the textbook the more rapidly will it be affected by the progress of current research.

The book is well-organized chronologically, and the treatment wisely takes the survey down to the beginning of the modern era (the BC - AD interface) for Europe as a whole. The emergence of the civilization of classical Greece thus falls within its scope, together with that of the Etruscans of Italy. This presents problems of treatment, which many earlier authors have avoided, since of course the availability of written sources brings Greece out of prehistory and into the light of history at that time, and the summary offered here will no doubt find its critics. But the solution is a bold one, and I find it altogether effective.

Minor faults and failings in several places can be rectified in a second edition. Meanwhile it is a pleasure to salute the appearance of a survey which for the first time since the New Archaeology of the 1960s presents the prehistory of Europe in a coherent and satisfying way. It will at once take its place as a new standard work, for it is quite simply the best introduction to European prehistory which is currently available. □

Colin Renfrew is Disney Professor of Archaeology at the University of Cambridge, and a Fellow of St John's College, Cambridge.

Fossil selection

W.S. McKerrow

Atlas of Invertebrate Macrofossils.

Edited by John W. Murray.

Longman/Halsted: 1985. Pp.241. Pbk £13.95, \$24.95.

IT IS very seldom that I have opened a new book with such delight. About half of its pages consist of magnificent photographs of well-preserved fossils, and primary congratulations must go to the photographers who have so ably produced these illustrations. The preface informs us that there are about 3,000 named minerals, but that the invertebrate macrofossils include tens of thousands of genera and hundreds of thousands of species. It has never been easy to select what to teach from this myriad. Now we have a choice of 900 well illustrated fossil genera, more than enough for an undergraduate course. Some of my favourite genera are missing, but most are there; more significantly only one or two minor groups of macrofossils are absent: hyolithids and archaeocyathids seem to have escaped the editor's net. (Vertebrates and microfossils are also absent, but by deliberate decision. Do they warrant two more books along the same lines?)

Seven of the contributors are from

British universities, five from the Natural History Museum, one from the Geological Survey of Greenland and one from Brigham Young University, Utah. They all know what is needed to give authoritative descriptions of the common fossil groups. Although there is some bias towards north-west Europe, the fossils illustrated and described include genera from all over the world. So the book will be a useful text for any student in any country who can read English, while the diagrams and photographs should even prove helpful to those with only a scanty knowledge of the language.

Most chapters include a glossary of descriptive terms, and also an evolutionary diagram showing the relationships of the larger taxonomic groups. But, as the editor points out (p.2), "taxonomy is not the main aim of palaeontology. It is the application of correctly identified fossils to the problems of biostratigraphy and palaeoecology which makes palaeontology a major part of geology". The teacher who uses this book in class will still have plenty of work to do, especially in explaining the biology, ecology and evolution of these extinct animals. However, this atlas will make his task (and that of the student) so much more pleasant and so much more efficient. □

W.S. McKerrow is a Lecturer in the Department of Earth Sciences, University of Oxford.

A view of solids

R. Rudham

Solid State Chemistry and its Applications.

By Anthony R. West.

Wiley: 1984. Pp.734. £37, \$67.95.

HAVING carefully defined solid state chemistry as the synthesis, structure, properties and applications of solid materials, the author of this book is somewhat confined within a straitjacket of his own manufacture. Unfortunately, his definition precludes consideration of the important interactions between solids and gases or liquids. The fact that the book has nothing to say about adsorption and heterogeneous catalysis is perfectly acceptable, but when metal oxidation is similarly ignored one cannot help questioning the desirability of such a definition of solid state chemistry.

The non-mathematical approach adopted by the author contributes to the readability of his work, but inevitably introduces some shortcomings. For example, the law of mass action treatment given for lattice defect equilibria is less informative than that of statistical thermodynamics, where the compensating contributions to the overall free energy from defect formation and configurational entropy are directly seen. Nevertheless, the

chapters on structure and properties present an interesting and coherent account of these fundamental aspects.

What is less certain is the merit of the last four chapters on glasses, cements, refractories and organic solids, when serious consideration of diffusion processes and solid state chemical reactions has been omitted. In particular, the final chapter on the organic solid state seems something of an afterthought and in its brevity unjustly emphasizes the inorganic nature of the subject. To be fair, the early chapter on synthetic methods presents information on chemical reactions involving solids, but also injudiciously absolves the book from consideration of their kinetics. It is in this chapter that one of the major founders of modern solid state chemistry, Carl Wagner, receives his only mention.

West has written a remarkably readable account of solid state chemistry as he sees it, but who will read it? Both size and price will preclude its formal adoption as an undergraduate text, although it should make the recommended reading lists. Postgraduate students and other researchers will find it gently informative on areas other than those of their personal endeavours. The most probable readership will be non-chemists who have reason to enquire into the nature and physical behaviour of solids at the atomic level. □

R. Rudham is a Reader in Physical Chemistry at the University of Nottingham.

Different elements to chemistry

Jon McCleverty

Inorganic Chemistry: Principles of Structure and Reactivity, 3rd Edn.

By James E. Huheey.

Harper & Row: 1984. Pp.936. Hbk \$37.50; pbk £11.50.

Chemistry of the Elements.

By N.N. Greenwood and A. Earnshaw.

Pergamon: 1984. Pp.1,542. Hbk £75, \$120; pbk £19.50, \$34.95.

Structural Inorganic Chemistry, 5th Edn.

By A.F. Wells.

Clarendon: 1984. Pp.1,382. £75, \$98.

OF THE three books reviewed here, two are, to most inorganic chemists, old friends (Huheey and Wells), and one is a keenly anticipated newcomer. The books by Huheey (now in its third edition) and Greenwood and Earnshaw are intended as student texts, whilst that by Wells (a fifth edition) is primarily a work of scholarship and reference directed at one aspect of inorganic chemistry — the structure of crystals and solids of all types.

I have always been an admirer of Huheey's account of the principles of structure and reactivity in inorganic chemistry, largely because, like my students, I found it immensely readable and a nicely presented package of material for the core of the first two years of an undergraduate course. Typically North American in its approach, combining a distinct flavour of the physical chemist's view of chemistry, nonetheless it advances inorganic chemistry within a topic approach which makes the consumption of a wide range of facts both appetizing and digestible.

The early chapters deal with atomic structure, bonding models, chemical forces, acid-base chemistry and chemical behaviour in aqueous and non-aqueous media. It is gratifying to see a chapter on solid-state chemistry, although perhaps rather small in the light of the rapid growth of interest in the subject. Later chapters are concerned with coordination chemistry in all its aspects, organo-transition metal chemistry (including ritual obeisance to homogeneous catalysis) and bioinorganic chemistry. There are three chapters covering some descriptive chemistry of the d- and f-block metals, and of the halogens and rare gases. This is an improvement over the earlier editions, although there are, inevitably, still quite significant gaps. To some extent, however, the omissions are partly catered for in the excellent chapter on periodicity. The appendices contain a wealth of physical data, and discussions of nomenclature and of group theoretical symbolism and symmetry (although this is not used specifically in the text).

Greenwood and Earnshaw provide a wholly different menu of inorganic chemistry, returning in a sense to a factual presentation based on single elements or groups of elements following the periodic table. Thus the s- and p-block elements take up most of the first half of the book, and transition metals, reported by vertical groups, the second half. The only time that topics are discussed is at the beginning — the origin of the elements, isotopes and atomic weights, and chemical periodicity and the periodic table — and later with an account of coordination chemistry which precedes discussion of the transition metals.

Within each chapter, the properties of the elements and their compounds are discussed systematically, and aspects of solid state, organometallic and bioinorganic chemistry are referred to at the appropriate point. An unusual and unexpected feature is the inclusion in the chapter on carbon of a major discussion of metal carbonyl and hydrocarbon chemistry, particularly from the structure and bonding point of view. But by far the most interesting and novel feature of this book is the liberal presentation of facts and figures on the uses and potentially useful properties of inorganic compounds, information which is almost totally absent from any other major texts in this subject area. The danger of including such material is, of course, that it may rapidly become out of date, but that problem apart this is a fresh approach to the furthering of the relevance of inorganic chemistry which is to be welcomed enthusiastically.

It is not helpful to say which of the two books one might choose as the basis for a course of inorganic chemistry at the tertiary level. Huheey is certainly appropriate for the first two years of a British undergraduate curriculum, and I suspect that students would prefer it initially as a more helpful study guide. However, Greenwood and Earnshaw's book unquestionably contains more chemical information, and course instructors will be immensely grateful to the Leeds chemists for having reminded us all that facts are facts, whereas theories often reflect passing fashions and fancies.

Wells's *Structural Inorganic Chemistry* cannot be realistically regarded as a student text, although senior undergraduates will undoubtedly use it for reference. It seeks to present the structural basis of inorganic chemistry for chemists, and it succeeds. It is a little bit like an Irish stew — everything is included, cooked in a special and uniform way, making up a rather fine and nourishing dish.

The book is divided into two parts, the first of which deals with a number of general topics, including the properties of polyhedra, the nature of symmetry and repeating patterns, the way in which spheres of varying sizes pack together, and bonding in molecules and crystals. The second, longer part describes the structural

chemistry of the elements, based on the groups of the periodic table. Here oxides, hydroxides, aquo species, sulphides and halides receive considerable attention, but there are also chapters dealing with metal carbonyls, carbides and alkyls, the coordination chemistry of the later transition metals, and metals and alloys. The text is usefully referenced and there are formula and subject indexes.

In a subject as vast as structural inorganic chemistry, encompassed in some 1,400 pages of text, there are bound to be some topics which receive only cursory treatment. However, this is likely to worry only the crystallographer rather than the broader congregation of chemists, and the author is to be congratulated on assembling such an amount of useful information in such an accessible form. □

Jon McCleverty is Professor of Inorganic Chemistry at the University of Birmingham.

Organic affairs

John Mann

Advanced Organic Chemistry, Part A, 2nd Edn: Structure and Mechanisms.

By Francis A. Carey and Richard J. Sundberg.

Plenum: 1984. Pp.726. Hbk \$59.50, £56.53; pbk \$16.95, £16.19.

Organic Chemistry.

By G. Marc Loudon.

Addison-Wesley: 1984. Pp.1,451. \$39.95, £39.

The Third Dimension in Organic Chemistry.

By Alan Bassindale.

Wiley: 1984. Pp.242. Pbk £7.95, \$17.95.

Spectral Problems in Organic Chemistry.

By R. Davis and C.H.J. Wells.

Chapman & Hall/Methuen: 1984. Pp.121. Pbk £5, \$10.95.

LIKE Shakespeare's *Henry IV*, *Advanced Organic Chemistry* by Carey and Sundberg comes in two parts. Last year (*Nature* 308, 136; 1984) I extolled the virtues of the second edition of Part B, which is concerned with the methodology of organic synthesis. Part A deals with structure and mechanisms and, like its companion volume, is aimed at the advanced undergraduate or novice graduate student.

The style is modern, and both molecular orbital theory and the frontier orbital approach are used to good effect. There is a lot of physical organic information pertaining to the various reaction types, and other highlights include chapters on stereochemical principles and conformational analysis, concerted reactions and photochemistry. The numerous problems provided are well-chosen, and many contain data taken from the research literature.

Overall, the book complements Part B

most satisfactorily — the one providing the theory, and the other the practice. There is, however, a rival text of the same title by Jerry March, published by McGraw-Hill, a third edition of which is due later this year. March has become an essential purchase for many graduate students, especially for its vast collection of literature references. Carey and Sundberg's books are more readable and much better illustrated, but are not so well endowed with references. Any decision between the two types of book will depend upon personal preference and the size of one's bank balance (assuming that one book costs less than two).

At a lower level there are now so many books on organic chemistry that any new contender must offer something different in order to succeed. Recent innovations include two-tone illustrations, comprehensive chapters on spectroscopic methods and sections on "special topics". In his book, Loudon incorporates the first two of these, but spurns the last, somewhat gimmicky one.

Most books of this kind commence with the alkanes and introduce mechanistic concepts through a consideration of free radical processes. In contrast, Loudon believes that the "curly arrow" formalism and heterolytic processes should be used as an introduction to mechanistic organic chemistry, and I suspect that many would support this. Two-tone illustrations are used to good effect, not only for mechanisms but also whenever emphasis is required, as, for example, in the chapter on NMR spectroscopy. This appears early in the book, and stresses the practical uses (with lots of examples) rather than the theory. The links with life processes are not forgotten, so we find biological oxidation alongside oxidation of alcohols; ionophore antibiotics in with the ethers; and the mode of action of S-adenosyl methionine as an example of sulphonium chemistry. In fact the chapter on organosulphur chemistry is more extensive than in any other recent text of this kind, and reflects modern usage of these reagents. The same can be said of the account of heteroaromatic species. Other nice touches include examples of experiments, amusing historical anecdotes and end-of-chapter summaries.

There are, however, some important omissions — for example pericyclic reactions and organophosphorus chemistry — and there is a rather limited treatment of the strategy of organic synthesis. In these respects Streitwieser and Heathcock's *Introduction to Organic Chemistry* (Collier Macmillan/Macmillan 1981) is superior. Omissions apart,

Textbook supplement — prices

Where possible both dollar prices in the United States and sterling prices in Britain are given in the bibliographical details for each book. Export prices will generally be higher. If a dollar or sterling price is not cited, the book is not available in one of the two countries. However readers should check both price and availability of books before ordering.

Loudon's book is beautifully presented, has lots of interesting problems and is very readable.

The Third Dimension in Organic Chemistry by Alan Bassindale is not an easy read, but the effort is well rewarded. It is much more than a text on the shapes of carbon compounds, and covers all of stereochemistry including the dynamic aspects. The book is divided into three almost equal parts, and commences with a section on general stereochemical principles which takes the reader from a pre-university level as far as elementary conformational analysis. Part 2 deals with conformations of acyclic and cyclic systems and introduces the concept of chirality. This prepares the reader for the meatiest part of the book which covers the stereochemical features of the main reaction types. Substitution, addition and elimination reactions are discussed in detail, and there is also a chapter on pericyclic reactions. Finally, the concept of asymmetric synthesis is introduced and this is most pertinent since so much modern synthesis is concerned with the quest for stereochemically pure products. This chapter should perhaps have been longer,

however, with a few more practical examples, such as the use of metal enolates or chiral hydrazones in asymmetric synthesis. One other suggestion for the second edition would be the provision of a decent stencil for the chair conformations depicted! Otherwise, the book is an excellent introduction to stereochemistry and should become very popular.

This should also be true of the book by Davis and Wells, which provides 56 spectral problems at a cost of about 9p a time. Infrared, NMR (proton and ^{13}C) and mass spectral data are given for each compound, and in most cases an elemental analysis is also supplied. The answers are given in the form of references to *Beilstein*, the *Merck Index*, the *Dictionary of Organic Compounds* or to Aldrich's chemical catalogue. The spectra are reproduced well and the problems are both interesting and demanding; this book is especially welcome because interpretation of spectra (in particular NMR spectra) is nowadays central to most courses in organic chemistry. □

John Mann is a Lecturer in Organic Chemistry at the University of Reading.

Metabolic change

M.L. Sinnott

Essentials of Bio-organic Chemistry.

By R.W. Hanson.

Edward Arnold: 1984. Pp.208. Pbk £6.95, \$14.95.

OVER the past four or five decades, the application of chemical techniques and concepts to biology has proved one of the most fertile fields of scientific endeavour. Undergraduates in the biological sciences should now, ideally, have a firm grounding in those areas of chemistry germane to their studies: thus biochemists should understand the chemical logic behind metabolic transformations. Dr Hanson has taken "bioorganic chemistry" to mean the material necessary for an understanding of the chemistry of the transformations of organic metabolites *in vivo*.

The ground the book attempts to cover is very well chosen — a treatment of the properties of biologically occurring functional groups, uncluttered by the recondite technology of Synthesis, is followed by chapters on enzymes and coenzymes. However, in execution the text is a catastrophic failure with serious errors of fact and comprehension throughout.

For example, we read that "in general all positively charged electrophiles are more powerfully electrophilic than neutral compounds" (p.24); that ΔG^* ($\Delta G^\ddagger$) is "a measure of the energy which must be associated with a collision between A and B" (for reaction to occur) (p.28); that "oxidations are invariably associated with a

large negative value of ΔG° " (p.36); that, in the context of *aliphatic* diazonium ions, aromatic diazonium ions undergo nucleophilic displacements "for example, with iodide ions" (p.75); that "the 'entropic effect' of enzymes may cause rate accelerations of 10^8 " (not 10^8M); and that "electronic catalysis might contribute a further factor of the order of 10^2 " (p.154). Dr Hanson's treatment of additions to the carbonyl group (p.87 ff.) is innocent of any mention of general acid-base catalysis, his discussion of prochirality does not distinguish between enantiotopic and diastereotopic groups, and the concept of transition state analogues materializes without explanation from "application of transition state theory to . . . enzymes" (p. 164).

Although wholly inadequate in matters of substance, the text pays much attention to nomenclature and IUPAC names are used for small molecules (propanone for acetone, 2-amino acids for α -amino acids): of course this scientific Volapük is impracticable for large biological molecules, which are still called by the names people use. So we see "methanoic acid" on p.103 but "formyl methionine" on p.107.

The standard of scholarship of this text holds a moral for those of our political masters who wish to grind down more of the tertiary education system to a "teaching only" function. Even if an academic has the insight to identify a real educational need — as Dr Hanson has done — that need is most unlikely to be filled unless the academic has a substantial research presence in the area concerned. □

M.L. Sinnott is Reader in Bioorganic Chemistry at the University of Bristol.

Animals illustrated

D.R. Newth

Integrated Principles of Zoology, 7th Edn.

By Cleveland P. Hickman Jr, Larry S. Roberts and Frances M. Hickman.

Times Mirror/Mosby/Blackwell Scientific: 1984. Pp. 1,065. \$34.95, £38. **General Zoology, 6th Edn.**

By Claude A. Villee, Warren F. Walker Jr and Robert D. Barnes.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp. 992. Hbk £22.95, \$36.95; pbk £18.95.

EACH of these texts is about thirty years and half-a-dozen editions removed from its first appearance. Each is the work of three authors who acknowledge help in revision from many sources. Each is large and lavishly illustrated, devotes its largest single section to a systematically arranged account of the animal kingdom, but gives over half its total length to sections on behaviour, cell biology, ecology, genetics and physiology. Both sets of authors, in fact, have tried to include in one volume the minimum background of chemical and biological science necessary to make sense of any, or all, forms of animal life. In consequence they have had to be sparing in their descriptions of actual animals. The balance struck in both books is, I think, about right for texts at this level.

It would be surprising if many of the idiosyncracies, personal opinions on matters in dispute, or even the occasional lapses in matters of fact or of taste which endear the single-author book to the reader, could survive the editorial process in a middle-aged text produced for large numbers of undergraduate students. These losses are the price we pay for a gain in reliability and for a reduced danger of major omission. These two books, having set out with similar aims, have adopted broadly similar methods and have succeeded to much the same extent.

However the two are not indistinguishable. Hickman *et al.* is the more relaxed in tone, the more generously produced and, perhaps, the more willing to comment, from the zoologist's point of view, upon matters of public or lay interest. Thus the book ends with a chapter on animals in the human environment which gives a balanced but forceful treatment of the need to adopt conservation measures worldwide and without delay. Old-fashioned textbooks might mourn the extinction of species, but would hesitate to quote specific legislation and litigation in describing any one case. The story of the attempts to save the snail darter from the effects of building the Tellico dam may well serve to excite more interest in that unfortunate fish than it would ever otherwise have received.

Hickman *et al.* is the more colourful

book in the literal sense, using colour photographs freely and sometimes to pleasing effect. Whether or not this always adds to the value of the illustration is perhaps doubtful. It also evades the modern publisher's unhappy proscription of footnotes by allowing use of the wide margins of the printed page for "marginal notes" in which additional or peripheral matter can be added without stemming the flow of the main story. A similar, but less slightly, trick is used by Villee *et al.* who place such matter in "boxes" which can take up much of the page. This apart Villee *et al.* is altogether more reserved in presentation, both visually and in the text. It provides a businesslike account of its material, but must be faulted for a rather cavalier treatment of some important areas. Parasitology, the insects of economic importance, indeed the insects as a whole, and regeneration are cases in point. While my own preference would be for the more lively and imaginative approach of Hickman *et al.*, there is not a great deal in it. □

D.R. Newth was formerly Regius Professor of Zoology at the University of Glasgow.

Aspects of insects

M.E.G. Evans

Insect Biology: A Textbook of Entomology.

By Howard E. Evans.

Addison-Wesley: 1984. Pp. 436. \$32.95, £14.95.

Insect Physiology, 8th Edn.

By V.B. Wigglesworth.

Chapman & Hall/Methuen: 1984. Pp. 191. Hbk £15, \$35; pbk £7.95, \$15.95.

INSECTS are now studied as living animals rather than as stamps in collections, and this changed attitude is reaching student texts. Both of these books deal with insects as living organisms: their forms, functions and lifestyles. The first book is a fairly large general text, which concentrates particularly upon the behaviour, physiology and ecology of insects, whilst the second is a concise introduction to their physiology.

Some years ago, H.E. Evans wrote what was probably the best recent book on insects which was comprehensible to the general public (*Life on a Little-known Planet*, published by Dutton in 1968), so I received his textbook with great interest and anticipation. In fact, this is a multi-author work, as Evans has five colleagues as co-authors, but the style is consistent and a high standard of clarity is maintained throughout.

The 18 chapters are grouped into seven parts dealing respectively with structure and diversity, functions and development, behaviour, plant-insect relationships, relationships with animals, ecology and pest management. There is also an appendix on non-insectan Arthropoda

which is followed by a useful glossary. Each of the chapters concludes with a summary followed by "selected readings"; these include the most pertinent references, but might have been longer and more comprehensive. With few lapses, the text is written simply and informatively, and the illustrations are mostly straightforward line diagrams which are neatly boxed to separate them from the text. The traditional entomological preoccupations with morphology, taxonomy and diversity have been condensed into the first section to allow for expansion of the later parts.

Wigglesworth's book is a new edition of a well-established work; indeed, there can be very few authors who have produced a new edition of their book a full half-century after the original date of publication. It covers the traditional range of insect physiology by describing the operation of the various organ systems (many of which have been elucidated by Sir Vincent himself over considerably more than the past 50 years). Although similar to earlier editions, much new material has been incorporated, and more subheadings have increased the accessibility of different subjects in the text. The references have been updated and many new figures added, while the narrative is still admirably readable and comprehensible.

Insect Physiology was originally a pioneer in the Methuen *Monograph* series — little books which were a great boon to earlier generations of student biologists who lacked the huge diversity of modern textbooks. But even if the texts reviewed here are both good in their different ways, do modern students need yet more books, recycled or new? If an established text can be revised adequately, there is good reason to keep it. Wigglesworth's small (but expanding) book probably is most useful in providing an introduction to insect physiology for non-entomological zoologists, or to whet the appetite of entomology students at an early stage of their training. More advanced students will need his *Principles of Insect Physiology* (Chapman & Hall, 7th Edn 1972), or an equivalent expanded treatment.

A new entomology text must provide a fresh look at the subject in order to compete, and Evans has had the advantage of being able to take a contemporary viewpoint throughout. *Insect Biology* would provide an excellent basis for a modern entomology course taught as one of several subjects for a zoology or biology first degree. For a more specialist entomology course, morphological and systematic reinforcement would be necessary, as provided, for instance, by Borror, DeLong and Triplehorn's *An Introduction to the Study of Insects* (Holt, Rinehart & Winston, 5th Edn 1981) or Imms' *General Textbook of Entomology* (Wiley/Chapman & Hall, 10th Edn 1977).

M.E.G. Evans is a Senior Lecturer in Zoology at the University of Manchester.

Introducing biology

E.C. Cox & R.M. May

Exploring Biology, 2nd Edn.

By Pamela S. Camp and Karen Arms.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.591. £27.95, \$32.95.

Bioscope, 2nd Edn.

By Thomas A. Easton and Carl E. Rischer.
Charles E. Merrill: 1984. Pp.719. Hbk £16.95; pbk \$19.95.

Biology: The Unity and Diversity of Life, 3rd Edn.

By Cecie Starr and Ralph Taggart.
Wadsworth: 1984. Pp.697. \$29.10, £49.60.

Biology: An Introduction.

By Kenneth D. Johnson, David L. Rayle and Hale L. Wedberg.
Benjamin/Cummings: 1984. Pp.615. \$28.95, £25.15.

ALL four of these texts are intended for students who will probably take only one biology course and whose teachers are interested in covering the broad range of topics now typically found in standard texts for science majors. Three of them follow what many have come to accept as the preordained little-to-big (molecules to ecology) order of presentation. The exception is Camp and Arms (CA), whose book begins with evolution and ecology, and then proceeds to cells, genetics, plants and animals; this book is essentially a scaled-down version of the same authors' *Biology*, a well-written text for science majors.

With the exception of Easton and Rischer (ER), which has an unfortunate number of errors, these books are scholarly, accurate, up-to-date and well produced — Starr and Taggart (ST) excepted —, including thorough coverage of plant biology, and good discussions of human anatomy and physiology. Although intended for a one-term survey course, they treat the readers seriously, something we found lacking in similar texts when we reviewed this field several years ago. The books have a seriousness of purpose and intellectual rigour that is easily lost when authors try too hard to enliven their subject by making it trendy and "relevant" (and even here there remain lapses from grace, such as the photo in Johnson, Rayle and Wedberg (JRW) captioned "the rock star Johnny Winter is an albino").

ST is an extraordinarily well illustrated book, with figures and photographs that rival those in Curtis's *Biology* for clarity and sheer beauty. The other three books are illustrated well, but none comes close to ST. All the books end each chapter with review questions and a list of suggestions for further reading. These lists are a bit dated, idiosyncratic and exhibit the usual high coefficient of inbreeding with other introductory texts.

The evolution and ecology sections do

not strike us as the strongest parts of any of the four books. In this respect, none of them is up to the standards set by Purves and Orians in *Life: The Science of Biology*. There are, however, interesting differences among the four. JRW and ER illustrate speciation with the rather tired example of the beaks of Galapagos finches; CA uses the more compelling example of the Hawaiian honeycreepers; ST also uses the Hawaiian honeycreepers, but with an illustration that is more complete and more beautiful than that of CA. All four books discuss continental drift and its relation to past and present biogeographical patterns, but while the other three show the usual picture of continental drift over the past 200 million years, ST have a new double-page spread correlating the evolution of life with the location of the continents since the late Cambrian (540 million years ago). On the other hand, ST is the only one of the books to perpetuate the appealing but incorrect generalization that roughly 10% of the energy at one trophic level is transferred to the next (it is one of our pet peeves that this "law" is so long in dying). On balance, however, ST seems to have



Nerve fibres on muscle (x900). The picture is taken from *Biology* by Claude Villee et al., published by Holt Saunders/Holt, Rinehart & Winston.

made the greatest efforts to break out from the incestuous patterns — the convergence in use and reuse of familiar examples — that afflict most introductory texts.

All the books make efforts to incorporate recent advances, going beyond the molecular biology of the gene to the molecular biology of the cell. But the text fully bringing an abstract of Alberts *et al.*'s *Molecular Biology of the Cell* into the introductory classroom remains to be written.

More generally, it seems that if an elementary text is to give some idea of the great diversity of methodology and levels of analysis currently found in the biological sciences, it must sacrifice something in the telling. In the books reviewed here, what is lost is a feeling for the way in which science is done and for the elegance of many experiments. It seems to us that this is inevitable in a non-trivial book for a one-term course, where the emphasis is on scientific content. Whether or not this is how non-science majors should be taught has always been a matter for lively debate,

and could well be the subject of a different essay.

None of these books spends more than a sentence or two on creationist theories. Only JRW includes a reference in the index, and the authors briefly and sensibly say that all such conjectures are matters of faith outside the realm of science. This represents a change in college texts over the past several years. Not long ago authors felt compelled to defend Darwinism against attacks from modern proponents of special creation (with Luria, Gould and Singer's *A View of Life* being a high-water mark). Happily, this is no longer so if these texts are at all representative. The poverty of special creation theories as scientific explanation is taken as given.

Broadly speaking, the intrusion of the social and moral concerns of textbook writers into purportedly objective texts must surely date from earliest times. In this respect the books under review also represent a change from recent crops, in that they mainly focus on science, tending to leave out issues that always have seemed to us more properly contained in social science texts. No doubt there will continue to be introductory texts on the market that grip the student with rhetorical pleas for the state of the environment or the brotherhood of man (or siblinghood of persons), but these are not among them. There are lapses, such as ER ending with the time-honoured two views of the future (replete with imaginative illustrations of how "the movement to space could begin with the construction of orbiting space colonies"), but in the main we have factual accounts that balance the many disciplinary calls on the student's attention with the need to remain comprehensive and easy to read.

All in all, we think ST comes out ahead. CA and JRW are both good, with JRW deliberately aiming a bit higher (for example, it gives the mathematics of the Hardy-Weinberg law, while CA does not). ER is pitched a bit lower than the other three, and contains quite a few serious errors and misleading figures: for instance, on page 115 the two polynucleotide chains of the double helix have the same polarity, while the codon table on page 117 is badly scrambled. We like ST best because it is so well illustrated, succinct and marvellously clear. It also manages to cover more ground and to do the best job of the three in the sections on energetics and chemistry, while retaining its comprehensiveness and avoiding excessive length and detail.

Personal preferences notwithstanding, all but ER are good value. This may in part be because the authors have worked closely with their reviewers, as acknowledged in each preface. The majority of the reviewers involved have wide experience teaching the students for whom the books are written. All of us profit from this collaboration. □

Edward C. Cox and Robert M. May are Professors in the Department of Biology at Princeton University.

Vertebrates as a whole

R. McNeill Alexander

Vertebrate Natural History.

By Mary F. Willson.

Holt Saunders/Holt Rinehart &

Winston: 1984. Pp. 621. £33.95, \$37.95.

"NATURAL history" is a term that needs rehabilitation. We tend to use it only to refer to amateur field studies, and in the names of museums, but it is an excellent, succinct description for studies that deal with whole organisms rather than tissues or cells. In *Vertebrate Natural History* Dr Willson uses it very broadly, to refer to subject areas ranging from environmental physiology to sociobiology. She tells us that "for the modern study of natural history, it is inadequate to construct a catalog of cute little tricks employed by organisms".

The book has four parts, which are not well integrated. The first is intended to set the scene, and includes elementary introductions to evolution and allometry, a review of the vertebrates (which is little more than a catalogue of orders, without anatomical detail) and a description of the world's climates and vegetation. The second part is the physiological one,

discussing senses, thermo- and osmoregulation, respiration, locomotion and migration, while the third (and shortest) deals with foraging and escape from predators. The final part covers such topics as home range and territory, courtship and mating, life history patterns and parental care.

In this book we are told about a great many aspects of the lives of a great many animals, but there is little cross-reference between one chapter and another and we are not given an integrated impression of the life of any species. The text is superficial, citing a great many investigations but providing little information about any one of them. Explanations are generally facile, without adequate reference to basic principles, and are sometimes misleading. The experimental evidence for statements is seldom given.

There are many fine drawings and photographs of animals mentioned in the text, and the chapters are headed by delightful decorative sketches, but few of the diagrams are original. In some compensation for the vagueness of the text each chapter includes a long list of generally well-chosen references — a remarkable number of the best papers of the 1960s and 1970s are listed, but only a few from the 1980s. □

R. McNeill Alexander is Professor of Zoology at the University of Leeds.

After Darwin

R.J. Berry

Evolution: A Modern Synthesis.

By W.H. Dowdeswell.

Heinemann Educational: 1984. Pp. 176. Pbk £6.50.

BIOLOGY first made sense to me when my A-level course reached evolution. The seemingly endless tread-mill of form and function, ethology and ecology, taxonomy and biochemistry suddenly became part of a coherent whole. I was fortunate in my teachers. Many students coming up to university have only a slight or garbled understanding of evolutionary biology; as a result they tend to believe that methodology-driven disciplines such as molecular genetics or behavioural studies are the real keys to biology. Anything that counters this false form of reductionism is good and it is a pleasure to see the appearance of Professor Dowdeswell's latest book.

Thirty years ago, Dowdeswell wrote a book called the *Mechanism of Evolution* in the Heinemann Scholarship Series. This was obviously a success because it went through several editions and reprints. His new book is presumably intended as a replacement. It includes chapters on the origin of life and the palaeontological record, but otherwise has a similar struc-

ture to the earlier work. But the new is not simply a rehash of the old: punctationalism is discussed, as are cladism and creationism (the last not entirely satisfactorily; but it is refreshing to find a fundamentalist correctly defined as one who adheres to the fundamentals of Christian faith), and odd snippets of information crop up which identify the book as written by someone who knows the subject rather than an intelligent hack who simply recycles existing texts. (For example, sickle cell haemoglobin homozygotes are conventionally regarded as having zero fitness, but Dowdeswell describes a Saudi population where fetal haemoglobin persists into adult life and increases the fitness of sickle homozygotes apparently to unity; and the influence of plant density on the frequency of primrose homostyles is described, taking the standard story of their maintenance away from mere speculation about the effects of inbreeding.)

Evolution is a text for sixth formers and for those undergraduates arriving at university with only a tenuous grasp of the subject. It is admirably designed for that readership, as would be expected from a teacher of Dowdeswell's experience. My only grouse is that it may be too placid: will it excite current generations of A-level candidates? I don't know; I can only hope.

R.J. Berry is Professor of Genetics at University College London.

An idea of primates

Michael Simpson

Primate Behaviour and Social Ecology.

By Hilary O. Box.

Chapman & Hall/Methuen: 1984.

Pp. 283. £10.95, \$22.95.

THOSE who enjoy working with and reading about primates for their intrinsic interest will welcome this book as a rich vein of information and reference. Here is an uncontroversial source to complement those primate behaviour courses which concentrate upon just a few strong ideas, illustrated with a handful of examples, described in depth. It is indeed useful to have a psychologist's synthesis of approaches to "intelligent" responses to changes within social and physical environments, evident in the sections "Responsiveness and Life Strategies", "Self-awareness", "Piagetian Techniques", "The Use of Tools" and "The Question of Culture". Other kinds of material, such as Packer's study of male transfer in baboons, are well summarized for teaching purposes.

However, two caveats should be made in response to the book's advertisement of itself as a text for students. First, strong theoretical lines are not pursued. It could be argued that in so far as the study of primate behaviour is in a "fact-gathering hypothesis-testing" phase, this book's unfocused Aristotelian approach is appropriate, and most of the current ideas available (including so-called sociobiological ones) cannot do justice to the complex data. But a particular viewpoint can also sharpen our focus on factual material, and there is already a harvest of particular facts whose quiddity cries out for theoretical support. Why, for example, should chimpanzees and bonnet macaques be almost the only multi-male primate groups in which males groom each other extensively? Why do the daughters of so many macaque families rank socially in inverse order of their ages? Theories answering such questions could have much to say about social competition and cooperation.

My second reservation is that students will not always find that closer reading clarifies difficult passages. For instance, gender ratio adjustments in Japanese macaques (p.120) and vervet monkeys (p.233) are mentioned, but the directions of such adjustments cannot be found in the text. Fig. 6.9 lacks a code for its letter symbols which would let it show "the situation clearly" (p.241). In some passages (for example p.43) the author is worsted in "the intolerable wrestle with words and meanings". □

Michael Simpson is in the Medical Research Council's Unit on the Development and Integration of Behaviour at the University of Cambridge.

Telling tales

Geoffrey Hall

From Darwin to Behaviourism: Psychology and the Minds of Animals.
By Robert Boakes.

Cambridge University Press: 1984.
Pp. 279. Hbk £35, \$69.50; pbk £15, \$19.95.

DISCUSSING the implications of his theory, Darwin wrote in the *Origin of Species* that "psychology will be based on a new foundation, that of the necessary acquirement of each mental power and capacity by gradation. Light will be thrown on the origin of man and his history". But the facts of psychology were to prove troublesome to Darwinian theory as he himself acknowledged in the *Descent of Man*: "the high standard of our intellectual powers and moral disposition is the greatest difficulty which presents itself, after we have been driven to this conclusion on the origin of man".

The problem for supporters of the "continuity hypothesis" was to find evidence for the existence of mental powers in non-human animals and this Darwin and his successors (notably G.J. Romanes and C.L. Morgan) set out to unearth. Their work forms one of the foundation stones of modern behavioural psychology and constitutes one of the major themes of Boakes' excellent book.

An earlier view of the nature of animal behaviour has its origins in Descartes' notion of them as reflex automata. The

second theme of the book is found in Boakes' discussion of the concept of the reflex as the mechanism of animal behaviour from its Cartesian origins, through la Mettrie, Hartley and the work of nineteenth-century German physiologists, to that of Pavlov and associates.

The two themes come together in the final third of the book, when we move to the early twentieth century and the scene of intellectual activity switches, for the most part, from the Old World to the New. In the United States we see the creation of an explicit behaviourism, a view of psychology which, to put it crudely, accepted a reflex account of animal behaviour but which avoided any conflict with evolutionary theory by its willingness to apply (or try to apply) the same account to human behaviour. Boakes, of course, does not put it crudely and his thorough discussion of behaviourism and its implications should help to counteract the simplistic and condescending descriptions to be found in so many textbooks.

Altogether this book is a fine achievement. There is no doubting the depth of scholarship but this is also a good story, well told, which should appeal to psychologists and philosophers not primarily interested in the history of their subjects. And everyone will obtain pleasure and instruction from looking at the illustrations, many of them from private sources and quite new to me, the discovery of which must have been a formidable undertaking in itself. □

Geoffrey Hall is Senior Lecturer in Psychology at the University of York.

Psychometrics at the trot

Steve Blinkhorn

Psychological Testing.

By John R. Graham and Roy S. Lilly.
Prentice-Hall: 1984. Pp. 433. \$39.10, £35.60.

ON FIRST acquaintance with psychological testing there is a constant demand from students for emphasis on details of published tests and their application. On the other hand lecturers may very properly feel that a sound grasp of psychometric methodology is of more value in getting to grips with the often difficult and tendentious issues associated with the use of tests.

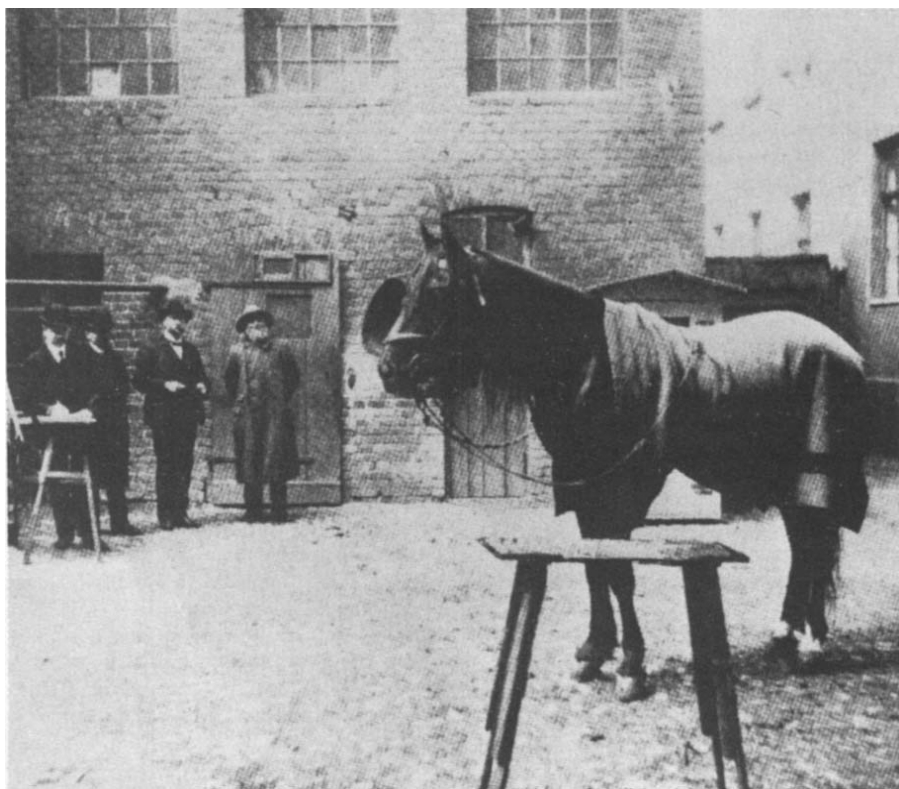
Graham and Lilly have resolved this tension in favour of the demand from below. After a quick, elegant canter round the basic statistical and mathematical concepts, the bulk of this book is given over to description and evaluation of published tests. The student is introduced to a range of issues via a discussion of particular tests, and appropriate primary sources are referenced. Concluding chapters present an overview of assessment as a process, and of controversial issues in the use of tests.

Within its self-imposed limits the book is admirably written, providing a smooth, well thought out account of most of the topics one would wish to include in an introductory course. This is a fundamental failing. There is no challenge left when every dilemma is polled, every contentious issue sanitized and a range of sound, sane opinion presented neatly gift-wrapped as if ready for instant regurgitation in a multiple-choice quiz.

This is not merely reviewer's dyspepsia: textbooks of this kind, for all their merits as digests, encourage amongst students the attitude that certain matters of opinion or judgement are matters of settled fact. A quick glide through classical test theory and reliability has been "done": now on to the serious business of discussing the value of ink-blots. As a result we have the all too common treatment of tests (referred to airily by acronym — a sure sign of the initiate) as the subject matter of psychometrics. This is the very opposite of the independent critical outlook one would wish to engender in those who may come to make use of tests. They may forget that those tested are people, and that test data do not have an independent existence apart from those people.

Non-American readers will find the list of tests considered parochial and occasionally out of date. They will also find the price beyond what it is reasonable to ask a student to pay. □

Steve Blinkhorn is Director of the Psychometric Research Unit and Senior Lecturer in Psychology at Hatfield Polytechnic, Hertfordshire.



Testing Clever Hans, the "calculating" horse that excited much attention early in the twentieth century. Hans's owner and teacher, Herr von Osten, is on the right of the group of men.

Different behaviour

Richard J. Cowie

Animal Behavior: An Evolutionary Approach, 3rd Edn.

By John Alcock.

Sinauer/Blackwell Scientific: 1984.

Pp.596. \$25, £19.80.

Biology of Animal Behavior.

By James W. Grier.

Times Mirror/Mosby/Blackwell Scientific: 1984. Pp.693. \$28.95, £32.50.

WITH new textbooks on animal behaviour appearing every few months, it is an achievement for any one of them to reach a second edition. John Alcock has gone one better, and his well established textbook is now appearing for the third time. What is more, revision of the previous edition has not just been cosmetic. The book has been largely rewritten and the material has been reorganized into a more logical sequence. John Alcock is a good writer, and like its

Besides these obvious omissions there are two further points which concern me. The first is Alcock's tendency to invent explanations in terms of evolutionary advantage for almost every behaviour mentioned, including many aspects of human behaviour. Whilst I recognize the temptation to do this, there is a danger that naive readers may accept these speculations as established fact. My second worry is Alcock's failure to present any of the more mathematical models which have been successfully used to predict how animals do behave. This is particularly true for the many optimality models, which the author praises for producing quantitative predictions (p.200) but fails to describe in any detail. However, despite these weaknesses — or perhaps because of them — I am sure the book will remain popular with many students.

Whereas Alcock's book is restricted in outlook but well organized and enjoyable to read, Grier's book is just the opposite. It is one of the most comprehensive textbooks on animal behaviour that I have seen, but the sheer volume of information and the order in which it is presented leaves one a little breathless and bemused. That is not to say that the book is badly written and in general Grier's summary of each topic is

good. He tackles many controversial subjects, such as the sociobiology debate, in an informed and balanced way.

Besides covering the more usual subjects, the author provides a crash course in both molecular and Mendelian genetics, an interesting historical perspective to the study of behaviour, and chapters on the observation and measurement of behaviour, abnormal behaviour, and applied behaviour, although these last two chapters are too simplistic to be of much use. The book is well laid out with many photographs and figures, the latter being generally well explained with lengthy captions, although some are confusing (for example those on p.42 and 43). There are numerous small details in which I would disagree with Grier, but overall I was very impressed with his encyclopaedic treatment of the subject and his evident grasp of many diverse areas of zoology.

If I were to choose one of these books to sit down and read for my own enjoyment, it would be Alcock's. But if I had to recommend one as a textbook to students it would be Grier's. □

Richard J. Cowie is a Lecturer in the Department of Zoology at University College, Cardiff.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Buffalo at home on the range in the Tsavo National Park, Kenya. The picture is reproduced from Ecology of Natural Resources by François Ramade, published by Wiley.

predecessors this edition is a joy to read. It is also well produced, and the layout, figures, photographs and binding are superb. A nice addition is a series of colour photographs on the inside of the covers.

Major changes to the content are the inclusion of a chapter on the development of behaviour and expansion of the section on sexual selection and mating systems. Both are improvements, but the book still has weaknesses which mar its usefulness as a general introductory text. These weaknesses stem from Alcock's interest in the evolution or function of behaviour, at the expense of a causal approach. For example, there is no mention in the index of such key concepts as motivation, ritualization or habituation, and the important topic of classical learning theory is dealt with in two short paragraphs under the sub-heading of plastic developmental systems.

Anything is possible

P.N.R. Usherwood

Neuroethology.

By Jeffrey M. Camhi.

Sinauer/Blackwell Scientific: 1984.

Pp.416. \$30, £24.

THE TASK of keeping abreast of developments in the biological sciences grows daily, even within the strict confines of one's own specialism. It was with some misgivings, therefore, that I approached this review, especially since my only credential was a brief love affair with invertebrate neuroethology in the late 1960s. However, my fears were unfounded since here is a book which is both highly informative and thought-provoking. Its aim is to introduce readers to the major concepts and research strategies which characterize neuroethology, a field of biology which continues to struggle for identity in an uncompromisingly compartmentalized world. Neuroethology remains an essentially schizophrenic experience, spanning neurobiology and ethology and making brave efforts to marry their languages and dogmas, but regrettably achieving little more, so far, than the connection of their fringes.

Neuroethology is essentially a book of two parts, one an introduction to the field, the other an account of nervous integration and behaviour. It is best when the author draws from personal research experience, one highlight being his description of cock-

roach escape behaviour and its neurobiological basis. My only criticism is that the emphasis is strongly anatomical and phenomenological with inadequate attention given to the advances in molecular neurobiology which have illuminated the past decade. There are good chapters on the visual and auditory worlds of animals, which contain essential background on physical factors in the environment, and useful accounts of sensory processing and motor control. Each chapter is concluded by questions for discussion and a good list of articles for further reading.

The author finishes, quite rightly, by marvelling at the complexity which characterizes the nervous systems of even "simple" animals and suggests, for example, that even after examining the circuit diagram of neurones in the leech we are left wondering how it works. This injection of mystery hints at a latent vitalism which denies the essentially mechanistic nature of neuroethology. He also lists six principles of neuroethology which he suggests have already been established and then argues that these are probably not global but restrictive and conditional. This lack of unifying principles is understandable in a fledgling science but it is unlikely to encourage the meek to enter the field. But Camhi has rekindled my interest in an old flame and all biologists with an interest in animal behaviour and nervous systems, simple and complex, will find his book well worth reading. □

P.N.R. Usherwood is Professor of Zoology at the University of Nottingham.

Fresh selection

T.R. Birkhead

Behavioural Ecology: An Evolutionary Approach, 2nd Edn.

Edited by J.R. Krebs and N.B. Davies.
Blackwell Scientific/Sinauer: 1984.
Pp.493. Hbk £25, \$42; pbk £13.80, \$25.

BEHAVIOURAL ecology has come a long way since the first edition of this book appeared six years ago, and is still moving fast. Some second editions are gentle updates, or ever so subtly improved versions of the first, but this is virtually a new book. There are very few chapters unchanged from the 1978 edition, the only exception being Maynard Smith's sex. Several of the chapter titles are the same as previously but often with new authors and certainly with a great deal of new information. As before the emphasis is on theory and the book is aimed at researchers and dedicated final-year students.

Behavioural ecology was born out of the cross-fertilization of ideas from several disciplines and it is exciting to find that the scope of *Behavioural Ecology* has expanded to include two additional topics, learning (Chapter 7 by Shettleworth) and plants (Chapter 14 by Charnov). While plants clearly don't behave as animals do, behavioural ecology theory has much to

offer botanists. Considering the rich rewards that so many zoologists have gained from the use of selection thinking, it is somewhat surprising that botanists have lagged behind. Charnov's chapter deals with sex choice, breeding systems and the conflicting interests of males and females. These are not the only areas of common ground for zoologists and botanists; others (at Sheffield and the University of East Anglia) are already looking at the foraging behaviour of plants!

In Chapter 12 Emlen updates his review of cooperative breeding — clearly a necessity if the number of birds known to breed in this way is any measure of this subject's interest. In the first edition Emlen noted that there were 150 known cooperatively breeding birds: the figure is now 300! The diversity of such breeding systems has also increased but at a much faster rate than our understanding of how they evolved. Cases in which close kin act as helpers are relatively easily explained, but why unrelated individuals should help is more problematical. Emlen discusses reciprocity, but another possibility, not yet fully explored, is that helpers mate with breeding females and actually help to rear their own young.

All in all this is an excellent text. It is well produced, clearly written and provides plenty to think about. □

T.R. Birkhead is a Lecturer in Zoology at the University of Sheffield.

Wholesome ecology

Robert H. Smith

Ecology: Principles and Practice.

By W.H. Dowdeswell.
Heinemann Educational: 1984. Pp.312.
Pbk £7.50.

Concepts of Ecology, 3rd Edn.

By Edward J. Kormondy.
Prentice-Hall: 1984. Pp.298. Hbk
\$29.65, £27; pbk \$22.90, £20.85.

Principles of Ecology.

By R.J. Putman and S.D. Wratten.
University of California Press/Croom Helm: 1984. Pp.388. Hbk \$22.50,
£22.50; pbk \$9.95, £9.95.

FEW textbooks that attempt to encompass the whole of ecology have received widespread acclaim. Odum's *Fundamentals of Ecology* (Saunders, 1953), Krebs' *Ecology* (Harper & Row, 1972) and Ricklefs' *Ecology* (Nelson, 1973) are the three which have been most often adopted as set texts, and all have run to second and third editions. Recently, the trend among publishers has been to launch series of smaller books, each dealing with a specialized aspect of ecology. However, many lecturers like to recommend a single book for purchase by first-year students which will also provide an introduction to later, more specialized courses.

Of the three books reviewed here, two are aimed firmly at the undergraduate market while Dowdeswell's *Ecology* is intended mainly for sixth formers and first-year university students. Dowdeswell's book is quite different from the other two and I shall consider it first. Dowdeswell provides a very basic introduction to ecological principles, going from physical factors through population dynamics to communities and ecosystems in less than 60 pages. No topic is considered in depth, nor is it intended to be. The book is written as extended notes with keywords highlighted in bold type and is clearly aimed at British, pre-university examination syllabuses. The second quarter of the book is about collecting and analysing data, and those involved in setting up simple field exercises for students may appreciate some of the practical tips. I do, however, have severe worries about the oversimplified treatment of statistics; for example, the statement "in order to avoid sampling error, the whole population should be collected but this is obviously impossible" does nothing to help the student struggling with the concept of the infinite population from which a sample is taken. The rest of the book runs through six groups of ecological communities and concludes with the obligatory chapter on ecology and man. I do not imagine that the level of description will be adequate for any biology degree courses, but the book is nicely produced

and will probably do well in the pre-university market at which it is aimed.

Kormondy's *Concepts of Ecology* is a middle-aged standard, first published in 1969 and possibly running to fat in an expanded third edition. My personal view is that his treatment of ecology is unexciting in both content and presentation. Most of the references are to books or general articles in *Science* and *Scientific American*; though adequate for first- and second-year undergraduate courses, final-year students need something more demanding. Just as Dowdeswell's book is parochially British, Kormondy's is parochially American and I could not find a single reference to European ecological journals: have *Oikos*, *Oecologia*, the journals of the British Ecological Society or *Nature* never published anything worth mentioning? Kormondy's approach is the standard one, dealing first with energy flow in ecosystems and biogeochemical cycles, then with population ecology and community organization, finishing off with human ecology.

Principles of Ecology by Putman and Wratten is the largest and most comprehensive of the three, with over 500 references, mostly to original papers. The order in which material is presented is the first feature that strikes the reader; after the usual introductory chapter on tolerance to abiotic factors and physiological and behavioural homeostasis, the authors launch straight into the ecological community as the unit of study. In some ways this unusual approach is a good one, since energetics and nutrient cycling are dealt with in an integrated way with species-abundance relations, community dynamics and succession. However, I did find that the links between different topics in the first four chapters are not made all that clear, and I reckon that students will have some difficulty in following the chapter on the niche without first thinking about intra- and interspecific competition (which come later). It is in the chapter on interspecific competition that the deficiencies of treating species interactions before single-species populations become most apparent. In order to develop a Lotka-Volterra model, exponential and logistic population growth models are skimmed through in a few lines, and the presentation of the conditions for coexistence is terrible.

Putman and Wratten place their chapter on population structure and analysis centrally — which of course means that the central concepts presented there are rather lost. The expanded repetition of exponential and logistic population models is again badly presented and likely to confirm the average biology undergraduate's prejudices about mathematical representation. A brief consideration of life tables leads into key factor analysis and the use of *k*-values but, like many authors, Putman and Wratten do not emphasize the direct link between the methods of analysis and implicitly assumed population models. Perhaps I am

being too critical of their coverage of topics with which I am most familiar, but I believe that it is extremely important to present quantitative concepts as clearly as possible without oversimplifying.

My final comment is about the way that evolutionary questions are dealt with in ecology textbooks. Kormondy hardly considers such questions, though he mentions natural selection when discussing biotic potential — and in my opinion gets it badly wrong when he says “To remain in the game, it appears that for a decreased value of r compensations have been achieved by natural selection in increased generation time”. Putman and Wratten have chapters on evolution and adaptation, and coevolution. Again, I could raise objections to much of what is said, but the authors are to be commended for stressing the relationship between ecology and evolution — albeit rather late in the book. Theories of evolution and ecology have not been well fitted together in the past, but things look more promising now. In particular, I feel that life history theory forms an important link, and behavioural ecologists are at last considering the ecological consequences of their theories and tests of optimality. These are two of the most exciting developments in ecology in the 1980s, and one hopes it won't be too long before they reach the textbooks. □

Robert H. Smith is a Lecturer in Applied Zoology at the University of Reading.

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Living in salt waters

Michael M. Mullin

Biological Oceanographic Processes, 3rd Edn.

By T.R. Parsons, M. Takahashi and B. Hargrave.

Pergamon: 1984. Pp.330. Hbk £22, \$40; pbk £10.95, \$19.75.

Marine Ecological Processes.

By Ivan Valiela.

Springer-Verlag: 1984. Pp.546. DM 118, \$44.

Basic Marine Biology.

By A.A. Fincham.

Cambridge University Press: 1984.

Pp.157. Hbk £20, \$36; pbk £7.95, \$14.95.

Marine Biology.

By H.V. Thurman and H.H. Webber.

Charles E. Merrill: 1984. Pp.446. \$28.95, £14.95.

ONE criterion in assessing the usefulness of new textbooks on marine ecology is whether recent findings, such as the hydrothermal vent communities, or renascent subjects, such as bacterial and protozoan ecology, are treated but also kept in perspective. Also important is whether the book covers other aspects of contemporary marine science — plate tectonics, mesoscale oceanographic fronts and eddies, interannual variability in oceanic climate and so on — in explaining marine biological phenomena. Finally, issues in general ecology, such as the role of disturbance in structuring non-equilibrium communities or food-limitation versus predator-limitation of population sizes, should be surveyed.

In the most strictly oceanographical book of the four reviewed here, Parsons and colleagues retain the “food web” emphasis of their earlier editions. They explicitly ignore intertidal and coral communities (and hydrothermal vents), and do not discuss sampling equipment. About 30% of the references are new and there are some new illustrations, for example of plankton at fronts, planktonic life cycles, isotopic ratios and how these can reveal food sources, and effects of pollution. To my mind, justice is not done to some new work (for example microbial heterotrophy), and the chapters on plankton seem to have been less thoroughly revised than the chapter on benthos. The concluding chapter on pollution and fisheries has been enlarged the most, and contains cogent accounts of why these problems continue to defy easy solution.

In its detail, emphasis on processes and citation of original literature this is a text for quite advanced students. As such, comments on the mismatches between what we can (and do) measure and what we need to measure would strengthen the early chapters. The authors do not shun

algebraic equations, though the level of mathematical treatment of different subjects is uneven. In the next edition, the authors could further emphasize processes by combining related subjects, such as secondary production or bacterial chemosynthesis, for plankton and benthos.

In his book, Ivan Valiela has done this admirably; each chapter presents the conceptual basis of an ecological process and examples from the water column, the benthos (including intertidal and estuarine habitats) or fresh water. Valiela summarizes physical or geological aspects only as related to specific ecological processes; his emphasis is very much on biotic interactions, and he discusses fisheries in this context rather than separately. He cites very recent studies, dares to speculate occasionally, and frequently indicates the observational and experimental evidence behind a generalization. The book is not easy reading, and supplementation by material from oceanographical sources may be desirable, but advanced students will find here a stimulating summary of modern marine ecology. I wished only for a fuller account of interannual variability in oceanic circulation and its effects on distributions.

Fincham writes for a general audience, and understandably devotes much of his book to marginal areas — the intertidal zone and estuaries — on which many human activities directly impinge and which are accessible to classes. There are useful diagrams of physical processes affecting biology (even sea-floor spreading) and many drawings of organisms, though several illustrations are superfluous. The focus is upon organisms and their interactions with the physical environment; more abstract concepts of food webs and communities are briefly treated as qualitative and static generalizations. There is the expected account of fisheries, but also unusual chapters on behaviour and biogeography which introduce such recent evolutionary terms as reproductive strategies, kin selection and altruism (though I believe these concepts are less representative of marine ecology than is, for example, the cycling of nutrients). There are good drawings and descriptions of equipment from a historical perspective, but Fincham does not elaborate upon the sources and strengths of evidence. The discussion of the intertidal zone, for instance, could easily have illustrated the value of manipulation by removal, transplantation or caging of organisms.

The diversity of illustration, ranging from sketches so crude as to be useless to a section of colour photographs, impressed me in the massive text by Thurman and Webber. There are also “study aids” — end-of-chapter exercises and appendixes, the latter including the metric system, taxonomy, a glossary and a dictionary of prefixes and suffixes. Over a hundred pages are devoted to organisms *per se*, and many aspects of the environment are

treated in similar detail, all without the mixed blessing of citations from the original literature. The authors introduce some very recent work, biological and otherwise, and also give a taste of how contemporary oceanography is done. Insertion of brief essays on special topics, including a couple of speculative interviews, varies the pace for the reader.

I continue to prefer the drier, academic style of Parsons *et al.* and Valiela. Students who are mature enough to appreciate the enormous wealth of information presented by Thurman and Webber should also be curious enough to check original sources for points of evidence occasionally, and should be willing to cope with concepts in a mathematical as well as a descriptive form. □

Michael M. Mullin is a Professor of Biological Oceanography at the Scripps Institution of Oceanography, La Jolla, California.

Ecological thoughts

John Lawton

Community Structure and the Niche.

By Paul S. Giller.

Chapman & Hall/Methuen: 1984.

Pp. 176. Pbk £5.95, \$11.95.

Predation.

By Robert H. Taylor.

Chapman & Hall/Methuen: 1984.

Pp. 166. Hbk £17.50, \$39.95; pbk £8.95, \$17.95.

PREDATION and interspecific competition are key ecological processes, although there is currently profound disagreement about their relative importance in structuring communities. Both phenomena have also spawned a diffuse and specialized literature that students and teachers alike find hard to break into. There is therefore considerable scope for good, simple introductory texts on both topics.

Giller's book has as its main theme the organization of communities, in particular the processes that control the numbers and relative abundances of species. It ranges widely to include discussions on predation, species-area relationships and food webs, although the dominant theme throughout is the concept of the ecological niche, as conceived by G.E. Hutchinson, nurtured by R.H. MacArthur and subsequently embraced by a whole generation of ecologists. In contrast, Taylor focuses on two- or three-species interactions, with a clear emphasis on mathematical rigour, testable hypotheses and the continual interplay between theory and experiment. Only in the last, short, chapter does he look at predation in communities, drawing the conclusion that "students of whole communities will need to be satisfied with a descriptive approach to static . . . patterns" — more or less what we find in Giller's book.

In general terms, therefore, the books make an interesting pair, with the potential at least to introduce students to a wide range of ecological problems and ways of thinking. Both are written for second- or third-year undergraduates, and postgraduates, with Giller's perhaps more accessible to novices and Taylor's decidedly more interesting for research ecologists of any age.

Specific details aside, I have some personal doubts about whether *Community Structure and the Niche* truly catches the spirit of its subject matter in the mid-1980s. Giller undoubtedly tries to explain controversies, and to draw attention to the uncertainties, doubts and problems that abound in this area of ecology. But he still left me with the impression that in spirit and philosophy the book could have been written in 1975. Moreover, I frequently found myself disagreeing with his main conclusions and interpretations, whilst in certain places crucial literature was ignored. A number of other things about the book also reduce its value as a teaching text. The 326 references appear in citation order (as in a letter to *Nature*), making it very difficult to follow the work of particular authors. More seriously, I frequently felt that the text was so packed with hurried references that many ideas would be unintelligible to all but the initiated. This fear was confirmed when I tested several sections on a brighter than average tutorial group. They found them cryptic and baffling. A slightly longer text, with less frenzied, fleeting allusions to everything that might be relevant would undoubtedly have made a better book.

No such criticisms can be levelled at Taylor. He explains theory and experiment with a lucid, direct style. Predator-prey interactions, ranging from protozoa, through insects, to birds and mammals, are mainly described using differential equations, but not in a way that should put off students — the mathematics is not very hard, and is made more palatable by plenty of graphical interpretations. I was particularly impressed by Taylor's careful discussions of why ecologists find it useful to construct mathematical models, and by his repeated attempts to highlight problems and difficulties, both in the construction of tractable models and in their empirical tests. In short, *Predation* should instil in young ecologists a way of thinking about problems that will be valuable whatever ecological processes they go on to study.

Predation may or may not be more important than competition as a force structuring ecological communities. Perhaps the next generation of ecologists will find such comparisons, and questions of relative importance, boring. But while we are training them to decide, I have no doubt that *Predation* provides more insights and a better foundation than *Community Structure and the Niche*. □

John Lawton is a Reader in the Department of Biology at the University of York.

Southern histories

Barry Cox

Vertebrate Zoogeography and Evolution in Australasia.

Edited by Michael Archer and Georgina Clayton.

Hesperian Press, 65 Oats Street, Carlisle, 6101 Western Australia: 1984. Pp. 1,202. A\$55. Post and packing A\$10 inside Australia, A\$14 overseas.

THIS is not so much a coffee-table book as a coffee-table in itself (area 650 cm², weight 3kg). Sixty authors have contributed 80 papers; most are concerned with Australia, but 13 deal with New Guinea, New Zealand and Lord Howe Island.

For Australians interested in biogeography and evolution, the trouble is that most books on these subjects treat Australian examples as a sort of awkward appendage, tacked on as a divine afterthought to the rest of Creation — a sabbatical daydream in which evolutionary fag-ends (for example monotremes) or pseudo-mediaeval beasts (kangaroos, wombats, moas and so on) were conjured up, their bizarreness emphasizing the sturdy normality of Northern Hemisphere animals, and their almost total lack of an evolutionary history teasing the poor palaeontologist. Australian students, in particular, have long needed a book centred on their part of the world. Well, here it is.

The first 13 contributions provide the background information relevant to Australasia in such topics as plate tectonics and palaeogeography, ecological zoogeography, the history of the climate and flora of Australia, vicariance and dispersal biogeography, and systematics. Each of the following sections on fish, amphibians, reptiles, birds and mammals commences with an account of the general evolutionary history of the group and ends with a checklist of the Australian representatives. The remaining contributions (a few being reprints of original research papers) deal with aspects of the zoogeography, evolution, ecology or discovery of Australian members of the group, or with the effects of man upon them. The book contains many figures and half-tone illustrations, and a few colour plates. The text is sometimes lighthearted in tone, and is also enlivened by occasional cartoons.

Though the quality and depth is inevitably a little patchy, this is in general an excellent student textbook. Even adding A\$14 for postage and packing, it still works out at about 0.04p per page. So students should buy it now, use it as a reference book until it's out of date — and then have a ready-made doorstop. □

Barry Cox is Head of the Department of Zoology at King's College, University of London.

Biotas in space and time

Carolyn M. Harrison

Biogeography: An Ecological and Evolutionary Approach, 4th Edn.

By C.B. Cox and P.D. Moore.

Blackwell Scientific: 1985. Pp.236. Hbk \$19; pbk £9.80.

Themes in Biogeography.

Edited by J.A. Taylor.

Croom Helm: 1984. Pp.404. Hbk £25; pbk £12.95.

WHEN the first edition of Cox, Healey and Moore appeared in 1973, it quickly established itself as a popular text and successive editions have served to reinforce its appeal. In this new edition, the authors have retained the essential structure of the immediate predecessor, and have expanded two chapters to discuss more fully how the major patterns of biota emerged in the distant and recent past. They do this with the distinctive blend of description and analysis adopted in earlier editions and the student is left in no doubt about the difficulties of reconstructing palaeoenvironments and their associated biotas.

The evident emphasis on analysis of the spatial response of biota to plate-tectonic movement and climatic change will be welcomed by many, but it has been achieved at the expense of any real discussion of how plants and animals adapt to their changing environments. In earlier editions, a chapter on natural selection, inbreeding and adaptation formed an important functional link between the evolutionary and ecological perspectives that the book seeks to forge. The omission of these topics in the new edition is not unexpected, given the complexity of evolutionary and ecological genetics; nevertheless their absence merely serves to reinforce a retreat from the stress on ecology that can be detected in the earlier editions. The task of preserving a balance among differing views of biogeography is a challenging one and it is easy to be dismissive of the value of ecological studies for students who are interested in the history of distinctive floras and faunas. But, for those large numbers of biogeographers who are intrigued by the impact of human activities on the distribution and survival of biota when seen as resources and cultural symbols, an ecological perspective is just as fundamental as an evolutionary one. As a result students in biology and geology departments will be better served by this new edition than will their fellows in departments of geography and environmental studies.

The tensions provoked by the position of biogeography at the meeting point of several disciplines is nicely revealed

through the 11 essays edited by J.A. Taylor. This volume is addressed to the advanced student and aims to provide a representative selection of traditional and emergent themes in biogeography, as seen by British researchers. The result is something of a potpourri. In these essays biogeography emerges as a multifaceted subject and as one to be approached in a variety of ways and with a range of analytical tools. Each essay stands on its own; most are scholarly, informative and up to date; some are idiosyncratic and a few are dull. Of more concern than the disparate nature of the assembled topics, however, is that here an opportunity has been missed. Many researchers will be disappointed to find that the chance to raise the level of debate about research priorities was not grasped more fully. Moreover, aspiring researchers will find little to help them decide which of the

several methodologies or techniques available are the most appropriate for tackling particular problems. This is not to belittle any one of the individual contributions to the volume, but rather to point to the benefits that a more obvious and focused discussion of research developments would have brought.

Biogeography has experienced something of a renaissance in recent years and some of its popularity can be attributed to the well-deserved success of books such as Cox and Moore. I cannot help feeling however, that students who are inspired early on in their careers by these authors, will not find their enthusiasm fanned equally by a reading of Taylor's volume. And that is a pity. □

Carolyn M. Harrison is a Lecturer in the Department of Geography at University College London.

Change in population

Michael B. Usher

Population Biology: The Coevolution of Population Dynamics and Behavior.

By John Merritt Emlen.

Macmillan, New York/Collier*

Macmillan, London: 1984.

Pp.547. £31.50.

How would most population biologists answer the question "how much mathematics should a student know?" Dr Emlen leaves the reader in no doubt since his book commences with a mathematical introduction, the "tools of the trade". The tools largely consist of matrix algebra, with, in addition, complex roots, variance and covariance, partial derivatives and the Taylor expansion. After this explanatory introduction it is odd that these mathematical concepts, especially matrices, are not made more of in the subsequent text.

Most of the topics that one would associate with a population dynamics textbook are covered — populations with and with-

out age structure, predator-prey interactions, competition and multi-species interactions — though it is, perhaps, surprising that parasitoids and mutualism are hardly mentioned. The book starts to diverge from the usual text when a strong genetic flavour is introduced in chapters that look at the genetic structure of populations and analyse models developed to deal with population parameters, either at a single locus or at multiple loci. Fitness is considered, largely by posing questions that raise a multitude of other questions: the same may be said about clines in colour, and their possible relation to environmental gradients.

The basic material having been covered, the evolution of various traits of population dynamics is then analysed in the final seven chapters. These traits include population parameters, age structure, mating systems and competitive interactions. The account of the latter is particularly concerned with niche separation, and emphasizes the coefficients proposed for measuring the effect of one species on another. The chapter dealing with predator-prey relations reviews the evidence for adaptations of both prey and predator, and for their coevolution.

The subtitle is somewhat misleading, since "behavior" is so underplayed in the text and the emphasis is firmly upon genetics; a subtitle such as *The Integration of Population Dynamics and Population Genetics* would have been much more appropriate. The book itself is almost dauntingly large, with large pages of small type, but it does encompass most of the subject, including reviews (to 1982), and incorporates discussion of many recent concepts. Although the style of writing seldom makes the text particularly lucid, I suspect that I shall make good use of this book for reference. □

Michael B. Usher is Reader in Biology at the University of York.

*Where appropriate, the price of a book in both Britain and the United States is printed at the head of a review in *Nature*. Prices are confirmed shortly before publication of a review.

It is apparently the policy of Macmillan Publishing Co. Inc. of New York (an entirely different company from Macmillan in Britain, although, confusingly, Macmillan in Britain will from 1 May 1985 distribute Macmillan Inc. titles in Europe) not to divulge prices of their books over the telephone except on the authority of "the vice president and director of marketing".

Attempts to persuade Macmillan Inc. to make public the dollar prices of *Population Biology* and *Microbiology*, 4th Edn (see p. 49) have failed, and these prices therefore do not appear here. The company might consider whether this policy is in its own best interest or that of potential purchasers of its books.

Parts of phycology

Howard Pearson

Introduction to the Algae, 2nd Edn.

By Harold C. Bold and Michael J. Wynne.

Prentice-Hall: 1985. Pp. 720. \$53.95, £49.15.

UNDERGRADUATES requiring an up-to-date text on the algae, which concentrates on cell structure, reproduction and classification, will benefit greatly from this factual, well-written, concise and well-presented book. Researchers who spend most of their time "torturing" the physiology and biochemistry out of algal cells, but want to know how their "tame" species fit into the greater scheme of things, will also profit from reading it.

The book was not written to cover all aspects of modern phycology (a point acknowledged by the authors), but to complement other texts on algal ecology, physiology, biochemistry and genetics. In this respect it is not adequate for an introductory phycology course, or for students attracted primarily to the algae by an interest in their potential biotechnological applications.

This second edition retains the format of

the first, with an outstanding introductory chapter (worthy in itself of publication as a booklet) preceding accounts of the algae division by division. The blue-green algae are included, as are new sections on *Prochloron* and algae of uncertain affinity. There are also other minor additions and changes to some of the keys. Mercifully, Bold and Wynne have dropped the unnecessary "phyco" from the algal division names so that, for example, the Chlorophycophyta revert to being the Chlorophyta. The glossary of terms is retained and aids the use of the simple-to-follow keys to the genera of each division, while the updated reference section provides a good lead into further reading. However, I regret the deletion in this edition of the very useful appendix on culture methods as the basic information greatly helps those embarking on the culture of algae.

In all this is an excellent book, full of useful pieces of information. The many photographs and line drawings complement the text and merit special praise. Bold and Wynne have managed to bring alive what even to many dedicated phycologists is a less than stimulating, albeit essential, area of the subject. □

Howard Pearson is a Lecturer in the Department of Botany at the University of Liverpool.

Eye to revolution

C.J. Leaver & S.M. Smith

Plant Molecular Biology.

By Donald Grierson and Simon Covey. Blackie/Chapman & Hall: 1985. Pp. 176. Hbk £17.95, \$39.95; pbk £8.95, \$17.95.

THE application of recombinant DNA techniques to the study of plant biology has led to the establishment of the new field of plant molecular biology. Yet it is only now that a book on the subject can be justified by the available data. In this short and up-to-date summary, Grierson and Covey have succeeded in conveying the present state of knowledge, as well as indicating the potential for genetic modification of higher plants which has raised hopes for a new green revolution.

The basic structures of plant genes are not dissimilar to those found in other organisms. However, the exciting questions in molecular terms arise from the presence in the plant cell of three interdependent genetic and protein-synthesizing systems, coupled with the unique developmental characteristics of higher plants.

The first chapter is a brief, obligatory introduction to recombinant DNA technology, which like the rest of the book would have benefited from the inclusion of a glossary to explain some of the necessary jargon. Subsequent chapters deal with the structure, organization and expression of

nuclear, chloroplast and mitochondrial genomes. The chapter on hormonal and environmental factors affecting gene expression reflects our level of ignorance in this important area, and is disappointing in its lack of coverage of some of the better understood aspects of the responses of plants to stress, such as heat shock and anaerobiosis. The accounts of plant-bacterial interaction in nitrogen fixation and in the natural gene transfer system of the *Agrobacterium* Ti plasmid are excellent introductions to each subject, as is that describing plant viruses, and lead logically into a final discussion of the prospects for genetic engineering of plants. This chapter illustrates the fact that although it is possible to list some of the goals for plant improvement, we still have little idea of the identity or mode of regulation of the genes we might modify or transfer.

The text contains errors, but this failing is partly compensated for by the up-to-date bibliography which allows ready access to original papers. The authors have produced a book which provides a solid basis for undergraduate courses on plant molecular biology, and which will also be useful to the inquisitive postgraduate and the informed entrepreneur requiring scientific background on the investment potential of plant biotechnology. □

C.J. Leaver is Reader in Plant Molecular Biology and S.M. Smith is Lecturer in Plant Biotechnology in the Department of Botany, University of Edinburgh.

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Chloroplasts, new and improved

R.P.F. Gregory

Chloroplasts.

By J. Kenneth Hooper.

Plenum: 1984. Pp.280. Hbk \$42.50, £40.38; pbk \$19.95, £19.

Chloroplast Metabolism: The Structure and Function of Chloroplasts in Green Leaf Cells, revised edn.

By Barry Halliwell.

Oxford University Press: 1984. Pp. 259. Hbk \$50; pbk £9.95, \$15.95.

IN THESE difficult times, when there is pressure to deliver courses that get to the point and stick to it, what usually gets left out is the sense of historical development of the present-day ideas, and all except the most sketchy treatment of comparative biology. It is a delight to find Hooper's text affirming at the very start his historical perspectives in a survey of the early stages of microscopy, chemistry and physics, and offering a challenging overview of the diversity of chloroplasts in algae as well as in higher plants. The work is well illustrated, with well-produced electron micrographs and line drawings. Equally welcome is the reappearance in paperback form of Halliwell's monograph; the reduced price will make this a more realistic prospect for the undergraduate.

The two books start off covering much the same ground, and present competent accounts of the structures, components and processes that make up the "light" and "dark" parts of photosynthesis. They also include practical details on such matters as chloroplast isolation which are likely to be found valuable by researchers and teachers.

From then on each author follows his personal interests. Halliwell is very strong on the interrelationships of photosynthetic metabolism, discussing at length (and with his practical interest) the pathways of C_3 , C_4 and CAM photosynthesis and their control, the roles of the chloroplast envelope and of its shuttle systems, and providing discussions of oxygen toxicity and polyphenol production. Hooper on the other hand presents a major coverage of his speciality of chloroplast genetics and protein synthesis, including the difficult

questions of the cooperation between the genomes and protein synthesis of nucleus and chloroplast (topics which Halliwell chooses to leave out except for a very brief note as an appendix). It is of course traditional to distinguish genetics and protein synthesis from metabolism, but this tradition is rapidly dissolving as the DNA workers prove themselves increasingly able to solve metabolic questions. Hooper concludes with a discussion of the evolutionary relationships of organelles and bacteria, and describes in some detail (and with further challenging questions) the work in progress on relating common sequences in proteins and in DNA, and so he returns to his initial theme of unity and diversity in this microcosm of Nature.

Halliwell's publisher is perhaps taking some licence in terming this a revised edition: there is in fact virtually no change of any substance (the pagination nowhere differs by more than one page from the original edition), and indeed Halliwell claims only to have made corrections and a few updates. He has provided some additional references to the literature, chiefly in the sections describing the Calvin

cycle and photorespiration, but he has not incorporated the recent advances in (for example) the oxygen-evolving system, nor in the chloroplast genome. Nevertheless within its terms of reference Halliwell's book still stands up well to comparison: for example, on the question of the transport of metabolites across membranes, he indexes both the C_3 translocator and the dicarboxylate translocator, and discusses their roles in the export of NADPH and ATP. By contrast Hooper is very brief here, referring only to the phosphate translocator in connection with triose phosphate export. Such key terms, including also transketolase and the names of important metabolites and enzymes, are printed in Hooper's text in bold type, but surprisingly do not always appear in the index.

Both books clearly have their strengths, and will be needed by the teacher of biology, biochemistry and botany. The student who is unwilling to purchase both will have to judge carefully where his interests lie. □

R.P.F. Gregory is Senior Lecturer in Biochemistry at the University of Manchester.

Green workings

J.D. Ross

The Physiology of Flowering Plants: Their Growth and Development, 3rd Edn.

By H.E. Street and Helgi Öpik.

Edward Arnold: 1984. Pp.279. Pbk £9.95.

Advanced Plant Physiology.

Edited by Malcolm B. Wilkins.

Pitman: 1984. Pp.514. Hbk £17.50, \$17.50; pbk £14.95, \$14.95.

Class Experiments in Plant Physiology.

By Hans Meidner.

George Allen & Unwin: 1984. Pp.169. Hbk £20, \$35; pbk £9.95, \$15.95.

WHEN they come to buy or use a book, the novice, the advanced student and the teacher have very different requirements. The beginner needs a broad coherent text which also brings out the excitement of the subject. The advanced student needs a detailed, balanced exposition and a lead into the current literature; whilst the teacher needs reassurance, a stimulus, and often a little help from his friends.

Perhaps the most difficult book to write well is the first, the "easy" one for the new student. In this third edition of *The Physiology of Flowering Plants* Helgi Öpik has based her text closely on successful earlier editions and concepts developed with her co-author, the late Professor Street. With minor flaws, she has succeeded admirably. The book flows through the subject with a nice style and conveys enthusiasm without avoiding uncertainties and controversies. Starting logically with seed imbibition and

germination we are quickly introduced to the interrelations of axis and storage tissues. The accepted dogma is outlined and then the criticisms are aired, for which the author must be given credit and respect for her feeling that the initiate should be exposed to problem areas. With emphasis on whole-plant physiology, we are led through photosynthesis, water relations and nutritional aspects in clearly written chapters.

An interesting account of stress physiology is then followed by discussion of the various aspects of growth and development. Again difficulties are not ignored; for example on page 184 there is a section headed "The problem of the mode of hormone action" (other texts do not even admit to a problem). This is exactly the treatment needed by the new undergraduate; it will inform and interest. Sadly, the diagrams and figures are small and mean, a major fault in many British publications.

In the preface to *Advanced Plant Physiology*, Professor Wilkins gives his opinion that a cooperative effort is necessary in compiling a text for the advanced student. This approach indeed gives strength in one sense. Unfortunately it is also the root of many of the book's weaknesses and the 20 contributions vary widely in treatment and usefulness. From my differing degree of enjoyment in reading the various parts I suspect that Wilkins with, perhaps, his colleague John Hillman could have written a more acceptable book on their own. Chapters such as theirs, on gravitropism and apical dominance, successfully put across ideas and experimental approaches in a manner suitable for their intended audience. It is at

Spring books

The next review supplement to appear in *Nature* is Spring books on 25 April.

The books to be reviewed include *Kapitza, Rutherford, and the Kremlin* (by Lawrence Badash); *The Problems of Evolution* (by Mark Ridley); *Time's Arrows* (by Richard Morris); *The Beginnings of the Nobel Institution* (by Elizabeth Crawford); *State of the World 1985* (by Lester R. Brown *et al.*); *The Broken Brain* (by Nancy Andreason); *Nuclear Winter* (by Mark Harwell); and "Surely You're Joking Mr Feynman!" (by Richard Feynman).

the student, not the researcher, that this text is aimed and balanced coverage is vital. This is not achieved in many of the contributions, although the editorial choice of subject divisions makes such a failing unavoidable.

The opening five chapters unashamedly tackle the "hormones", largely in a biased and sometimes dogmatic manner. The material contained here may indeed be recent, but the philosophy is still that of Frits Went. These accounts are not without scholarship, some being good reviews, but they deal with relatively narrow areas often of marginal relevance to normal plant growth. Also there are slips in terminology which annoy rather than confuse (although some are misleading, such as the use of "vernalization" for "stratification"; some plants can be vernalized as a seed, but this is a very different phenomenon to dormancy breakage).

I did not greatly enjoy this part of the book but found the rest of it more satisfying, albeit with some exceptions. The discussions of growth movements, rhythms, water relations and mineral nutrition are well constructed. Photosynthesis, however, is covered in a peculiarly unbalanced manner with aspects as important as C_4 metabolism and photorespiration being squeezed into the last two pages. Another odd component is the chapter on phytochrome — the undergraduate basing his studies on this is going to need guidance as, I suspect, will many lecturers. If this chapter is meant to be balanced by that following, on photomorphogenesis, it fails; here are the views, and largely the work, of one laboratory with little regard for other opinions. In contrast, the rather neglected phenomena of juvenility, photoperiodism and vernalization are well described and reviewed in a readable fashion, and are followed by straightforward accounts of germination, senescence and abscission.

Parts of this book are very good. But those using it must be supported by teachers unafraid to dispute the written word, so readily accepted by students.

Hans Meidner has written *Class Experiments in Plant Physiology* for those who teach the subject. Over the years a number of laboratory guides in plant physiology have appeared, usually emanating from the United States and written for students. These have not proved popular in Britain where there are smaller classes and different pressures. This time the collected experience of successful teachers and the author's attention to detail will be gratefully received. Class experiments in plant physiology, within the constraints of the timetable, are notoriously difficult. The suggestions, advice and hints contained here will be of great use to both the experienced practitioner and the new lecturer — if there are any out there in these hard times! □

J.D. Ross is a Lecturer in the Department of Botany at the University of Reading.

Membrane biology moves on

M.J. Selwyn

Membranes and their Cellular Functions, 3rd Edn.

By J.B. Finean, R. Coleman and R.H. Michell.

Blackwell Scientific: 1984. Pp.227. Hbk \$19.50; pbk £9.80.

MEMBRANE biology has advanced rapidly in the five years since the appearance of the second edition of *Membranes and their Cellular Functions*, a book which covers a course in membrane biology at first- or second-year university level more completely and satisfactorily than most of its competitors. A revised edition is therefore both welcome and timely.

Much of the material is included in that excellent tome *Molecular Biology of the Cell* by Bruce Alberts *et al.*, but this is less convenient because of its weight and because the information is scattered through the book. Robertson's *The Lively Membranes* is stimulating but very much slanted towards bioenergetics, while Harrison and Lunt's *Biological Membranes* or Houslay and Stanley's *Dynamics of Biological*

Membranes are pitched at a more advanced level.

This edition has grown in length and content, and the need for growth can be readily appreciated by comparing the chapters "Recognition, Response and Communication", "Secretion, Endocytosis and Lysosomes" and "Biosynthesis and Turnover" with their counterparts in the second edition. Together with a new chapter on membrane variations and membrane pathology, these are where the most expansion has been made, to include, for example, extensive descriptions of receptor mechanisms and the biosynthesis and processing of proteins which are incorporated into or pass through membranes. The chapters on membrane structure, and composition, and on ion and solute movements have also been thoroughly up-dated, but the treatment of the physical chemistry of ionic equilibria still lacks rigour.

The authors' style is readable and the layout — a central text with wide margins containing topic titles on one side, diagrams and equations on the other — makes for easy reading, browsing or reference. Overall this is an excellent volume, accurately written and attractively presented.

M.J. Selwyn is Dean and Senior Lecturer in the School of Biological Sciences, University of East Anglia.

To start with . . .

Roger Hill

Genesis on Planet Earth: The Search for Life's Beginning, 2nd Edn.

By William Day.

Yale University Press: 1984. Pp.299. Hbk \$49, £35; pbk \$14.95, £12.95.

THERE is no better example of the inadequacy of a multidisciplinary approach to an interdisciplinary problem than in the search for probable origins of life. Progress depends as much on the breadth of understanding and synthetic abilities of individuals as it does on the design of relevant experiments or on sagacious observation. Such qualities are demanded in equal measure from commentators on the field, and William Day is clearly competent to provide us with a useful review of current results, theories and speculations.

The conceptual dimensions of the subject call for an iterative development, and the repetition of subject matter in new contexts is a welcome feature of the book. For example, Day has chosen the fossil record as his first substantive topic, and the prokaryote-eukaryote distinction introduced here is fully described again after much compressed information on the cellular and molecular basis of life. After two chapters on bioenergetics, the reader is ready for a scientific presentation of the problem, and a review of prebiotic syntheses of present-

day biomolecules follows. The crux of the matter, the interface between prebiotic chemistry and biochemistry, is seen to be the abiotic polymerization of preformed nucleotides. Encapsulation by lipid bilayers and rudimentary photosynthesis gives life in slow motion before the development of protein synthesis and the appearance of enzymes. Extraterrestrial life is considered briefly, and the twenty-seventh of 28 short chapters is devoted to the organic analysis of meteorites.

Inevitably, subject specialists will find much to disagree with in the detail of this wide-ranging review, and many chemical evolutionists will regard as implausible the claim that not only are present-day polynucleotides essential prerequisites for life of any kind, but also that they could appear spontaneously as the outcome of a sequence of highly probable prebiotic syntheses. But there is much enjoyable and stimulating reading here. Day's historical and statistical perspectives, his descriptions of possible pre- and early-life scenarios and his speculations on the nature of future evolution are more than sufficient to encourage the reader to persist through the occasional conceptual thicket.

One caution to students: the author frequently presents conjectures in the style of factual statements which, though easing the narrative, calls for an assiduously critical presence of mind. □

Roger Hill is Reader in Chemistry at the Open University.

Our own inheritance

Ben Carritt

Human Genetics. By Elof Axel Carlson.
D.C. Heath: 1984. Pp.432. \$27.95.
Patterns of Human Heredity: An Introduction to Human Genetics.
By James R. Brennan.
Prentice-Hall: 1984. Pp.340. \$35, £31.90.

IT SHOULD not be imagined that a reviewer's heart beats faster with the arrival of a book based on a one-term course for non-science students. Moreover, chapter headings such as "Reflections on the Human Condition" (Carlson's first) fill the cynical and hard-bitten experimentalist with dread. And in Britain, where the contribution of science faculties to liberal studies teaching is negligible, it is tempting to dismiss such books as an irrelevancy. I was therefore pleasantly surprised by how much I enjoyed reading the first of the two reviewed here.

Carlson's *Human Genetics* is indeed intended as an introductory text for students with "no prior science course work", and the treatment of many important areas is therefore necessarily superficial. In some cases, additional depth is provided in the form of extensive legends to the excellent figures, and in others the details are contained in tables. At times, this device deprives the author of the space to explain his point with sufficient clarity: it also tends to give the impression that the transmission of scientific knowledge is not the primary purpose of the book. In a sense, it would be as well if this were the case, because viewed strictly as a genetics text it can be faulted. I thought, for example, that the coverage of chromosome abnormalities was too sketchy, and that there was too

much developmental biology. Neither could I see the reason for inserting an account of radiation damage as the third chapter when mutation is not covered until much later on.

Yet this is an immensely readable book, which seems to revel in the opening of controversy where almost any kind of biological principle is involved. IQ testing, the genetics of skin colour, the genetic basis of race and the "eugenics" programme of the Nazis are obvious inclusions, but there are many others besides. I particularly enjoyed the nuggets of useful statistical information, included in tabular form, such as the changing patterns of mortality, the causes of mental retardation in mental patients and so on.

Patterns of Human Heredity, aimed at a similar audience, is very much more a genetics text. The structure of the book is logical, proceeding through cell structure, DNA and chromosomes to Mendel, and then on to genetic diseases, polygenes and population genetics. The later sections of the book are well handled, particularly those which are mathematically based, such as polygenes and population genetics. One wonders, however, whether they might not be too advanced for a readership which is considered to be in need of the sort of detailed description of human reproduction which appears earlier in the book.

I found Brennan's use of language annoying and sometimes difficult to understand, but this may be a personal or a national difference in usage. Both he and Carlson provide review questions and summaries for each chapter — useful learning aids. Brennan also gives answers to his less discursive questions, which is helpful, if repetitive. □

Ben Carritt is in the Medical Research Council's Human Biochemical Genetics Unit, London.

Genetic similarities

D.J. Cove

Genetics, 2nd Edn.
By Charlotte J. Avers.
Willard Grant/Wadsworth: 1984.
Pp.644. \$27.50, £13.95.
Genetics, 3rd Edn.
By Ursula Goodenough.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.894. Hbk £24.95, \$37.95; pbk £13.95.

THIS is the third opportunity I have had to review the annual crop of introductory genetics textbooks for *Nature* (see *Nature* **289**, 702–704 (1981) and **295**, 476–477 (1982) for earlier reviews) and I will not repeat here in detail my views on how I believe genetics teaching should cope with the integration of the classical concepts of the subject with the explosion of knowledge that has taken place as a result of

molecular analytical techniques. This year's books are new editions rather than new texts and so it would be surprising if either offered any radical change in its teaching strategy. Avers remains an example of the "Mendel before molecules" approach while Goodenough is a "Let's start with DNA" book. Although the two authors have adopted what at first sight appear to be completely different teaching strategies, these books illustrate clearly how such overall strategies are rather unimportant. Each contains a chapter on Mendelian inheritance and there is little difference between them. Both stick faithfully to history, introducing Mendelism with Mendel and so, like most other textbooks of genetics, the student is required to cope all at once not only with segregation but also with diploidy and dominance. Both texts give the initial impression that each gene is represented by only two alleles, one dominant to the other, although Avers introduces multiple alleles and incomplete dominance much sooner than

Goodenough. In both books, Mendelian genetics is set in a sealed compartment with little reference to modern knowledge of gene structure or function.

Population and evolutionary genetics are also treated similarly in both texts. The subjects are dealt with last, in another compartment almost like an embarrassing appendix. Set against the excitement of recent advances in genetics at the molecular level, it is now often difficult to interest students in population and evolutionary genetics even though these topics remain of central importance to the subject. The integration of these topics into the general body of genetics teaching is therefore crucial.

As is to be expected, the most significant changes which these two new editions incorporate is in the area of molecular genetics and both include an up-to-date account of modern techniques for the handling and analysis of DNA and of the findings to which these new techniques have led. Here Goodenough, building on the strength of the molecular and microbial side of her earlier editions, has the edge.

The treatment by both texts of the regulation of the use of genetic information and of the genetic programming of development remains anecdotal. Perhaps the most important principle to emerge out of the study of the regulation of gene expression is that general principles are sparse. The diversity of regulatory mechanisms involved in the control of metabolism is not stressed enough in either book, and, in both, developmental regulation consists of the best stories told in an unrelated manner. Here Avers, perhaps because she has less space, achieves slightly more integration. Except of course in the treatment of Mendelian genetics, neither book gives much space to plants. Avers in her table of the DNA content of "representative eukaryotic genomes" includes no green plant! □

As our knowledge of genetics becomes more detailed, the subject becomes more challenging to teach especially in introductory courses. Conventional approaches become less satisfactory and integration more difficult since there is so much to be integrated. I believe that it is now best to begin such a course with a fairly complete but largely uncritical review of our current state of knowledge and then to deal with the experimental evidence which supports this overview, at the same time building flesh onto the initial skeleton. Neither of these texts adopt this approach. Both in fact offer us the same recipe as before and although they include all the necessary new ingredients the flavour is not much changed. If you liked either of these books before you should like the new editions more. If you are a first time buyer, however, I do not think they are among the best buys. □

D.J. Cove is Professor in the Department of Genetics at the University of Leeds.

Cells and the single author

J.G. Edwards

Cell Biology, 2nd Edn.

By Gerald Karp.

McGraw-Hill: 1984. Pp. 896.

\$35.95, £29.95.

Cells and Organelles, 3rd Edn.

By Eric Holtzman and Alex B. Novikoff.

Holt Saunders/Holt, Rinehart &

Winston: 1984. Pp. 660. £24.95, \$32.95.

THE acclaim which greeted the publication of *Molecular Biology of the Cell*, by Bruce Alberts and five other authors (Garland, 1983), was not solely a response to the excellence of its parts. As Joseph Gall pointed out in his review (*Nature* 302, 637; 1983), the inclusion of material on development, the immune and nervous systems and special features of plants, pointed to ways in which the teaching of biology at the cellular and molecular levels might be better integrated. In comparison, the narrower horizons of earlier textbooks may seem to do less than justice to the essential unity of modern biology, so one looks at their new editions for compensatory virtues.

Karp's book shares with Alberts's a much stronger component of molecular genetics than used to be found in texts entitled *Cell Biology*, but despite its new cover photograph of a myogenic clone still lacks Alberts's strong foray into developmental biology. Where both Karp and Alberts cover the same ground, however, the former more often manages to give some insight into experimental origins. For example, Alberts will tell you how many ribosomal genes there are in a *Xenopus* oocyte, but not about the anucleolate mutant; that allolactose is the real inducer of the lactose proteins in *E. coli*, but not about the diploids which helped unravel the system. Karp sometimes also has the edge in significant details (not just on matters such as oncogenes, where the passage of two years naturally makes all the difference).

In most areas with which I am familiar the reliability seems high, although I am unhappy about the way in which Karp presents the Hayflick phenomenon, both by discussion and juxtaposition, as the result of defective cellular processes. A balanced account would surely include the idea of programmed cell-death, and thus make better sense of immortalizing mutations. Karp's micrographs and diagrams (the latter now in two colours) are excellent. The whole is an impressive achievement, which runs Alberts much closer than I had imagined possible and will strongly tempt those of us who share the author's view of the importance of experimental history for current understanding.

The shorter *Cells and Organelles* is closer

in content to a traditional cytology textbook, and much gentler with the molecular aspects. Controversial areas get balanced, essay-like discussion and there are virtues of a reflective kind. You have to look in Part III of Alberts to understand Karp's cover picture, but only Holtzman and Novikoff point out that this revelation of the route to multinuclearity was one of the early triumphs of cell culture. Small unreliabilities disturb confidence, however: a diagram of skeletal muscle in which the muscle fibres are drawn to look like fibrils; another of a hepatocyte with empty space labelled for a microfilament; EGF named epithelial growth factor; pyruvate produced anaerobically from glucose. In the review copy a number of micrographs are much too dingy for clarity and impact, and some are patchily unsharp.

A reviewer should perhaps be wary of laying emphasis on how a textbook handles his own small favourite corner. Nevertheless, the time has surely come for better treatment than can be found in any of these volumes (including, surprisingly, Alberts) of the real progress which has been made towards identification of the molecules which join cells together. If any reader is busy revising another textbook, or wants to know about this topic for other reasons, my advice is to ignore most of the secondary literature, and give due attention to the strategy first adopted by Gerisch and also put to such good effect by Gerald Edelman and others. □

J.G. Edwards is a Senior Lecturer in the Department of Cell Biology, University of Glasgow.

DNA in sound and vision

E.J. Wood

The Techniques in Genetic Engineering Video Library.*

Series editor R. Williamson.

IRL Press: 1985. PAL in VHS and

Betamax, £85, \$170 each tape, £640,

\$1,280 the set. Supplementary charge for PAL in U-Matic and NTSC and SECAM standards conversions.

LAST year I reviewed the first videotape in this series (*Nature* 309, 566; 1984). Now that the whole series is available, how do the programmes live up to the initial promise of that first tape?

Each of the seven subsequent programmes concentrates on a specific technique or a limited area, and lasts 20–30 minutes. After the introduction of the presenter ("an expert in this field") by Professor Williamson, there is a brief and generally clear account by the expert of why a particular technique is of value, and usually some graphics are used to demonstrate in detail how DNA etc. is cut up and manipulated. These individuals mostly do not look directly at the camera, which I found somewhat annoying, while the level of background noise in some of the sequences is unduly obtrusive at times. These sections move fairly rapidly and would not easily be absorbed by the student at the first sitting. But then the video can be "stop-framed" or replayed, until the details are clear. The graphics themselves are quite well done, although they cannot

be said to use animation to its fullest extent, at least in the earlier tapes.

The tapes can be viewed in any order, although most people are likely to do cDNA cloning first. Several times demonstrations are repeated — for example, preparing DNA by ethanol precipitation, making cells competent by adding CaCl_2 , electrophoresis on slab gels and baking nitrocellulose filters in an oven. Maybe each is appropriate at that particular juncture, but given the cost of the whole set of programmes so many repeats were perhaps unnecessary.

A more general, but more serious point, is that in places too much detail is given about a technique, so that the underlying message becomes obscured; for example, it cannot really matter very much that the buffer reservoir of a slab-gel apparatus holds 0.8 litre of buffer, and excessive attention is paid to the disposal of radioactive pipette tips. Still, the demonstrations of techniques are indeed on the whole of high quality: the only exception to this is the demonstration of the microforge, which is poor but probably technically difficult to show.

In order to benefit fully from the tapes students would have to work through each a number of times, stopping and starting as necessary, probably writing down the sequences in the graphics. At this level I feel they would learn quite well from the tapes alone. The prize for the best and clearest tape goes to *Oligonucleotides*, closely followed by *Microdissection and Microcloning*, while the worst missed opportunity was in animating nick translation which could have been done so much better. But this must have been a difficult project to mastermind, especially to judge how much to put in and in what detail, and overall the tapes do succeed in their aim of being a "self-paced teaching aid". □

E.J. Wood is a Senior Lecturer in the Department of Biochemistry, University of Leeds.

* 1. *Nucleic Acids Techniques — An Overview*; 2. *Gene Analysis and Southern Blotting*; 3. *DNA Sequencing Using M13*; 4. *Gene Libraries*; 5. *Expression of Cloned Genes*; 6. *Oligonucleotides — Synthesis and Use*; 7. *In Vitro Mutagenesis and Use*; 8. *Microdissection and Microcloning*.

Recombinant limits

S.D.M. Brown

An Introduction to Recombinant DNA.

By Alan E.H. Emery.

Wiley: 1984. Pp.223. Pbk £6.75, \$17.95.

Genetic Engineering in Higher Organisms.

By J. Roger Warr.

Edward Arnold: 1984. Pp.58. Pbk £2.85.

The Genetic Code and Protein Biosynthesis, 2nd Edn.

By Brian F.C. Clark.

Edward Arnold: 1984. Pp.76. Pbk £3.20.

Bacteria, Plasmids, and Phages: An Introduction to Molecular Biology.

By E.C.C. Lin, Richard Goldstein and Michael Syvanen.

Harvard University Press: 1985. Pp.316.

Hbk \$35, £34.95; pbk \$18.50, £18.50.

From Cells to Atoms: An Illustrated Introduction to Molecular Biology.

By Anthony R. Rees and Michael J.E. Sternberg.

Blackwell Scientific: 1984. Pp.94. Hbk £12, \$12; pbk £6.80.

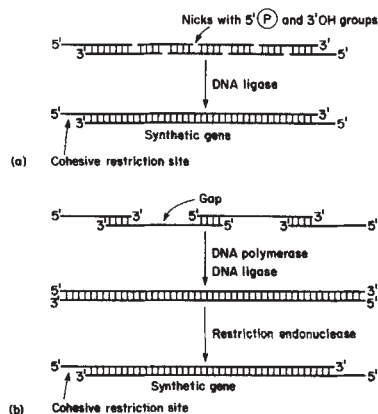
AS recombinant DNA techniques continue to stride forward there is an ever-growing movement to introduce this subject and its relationship to molecular biology at all levels of undergraduate teaching and in a wide variety of disciplines. There is also a strong demand for textbooks to cover the scope of the relevant techniques and the insights they have provided. But this need has disguised the fact that recombinant DNA is an "illustrator" of the systems it is used to investigate, and as a system *per se* has less intrinsic value; indeed, in many cases, the continued development of recombinant DNA techniques has depended upon the knowledge dissected from the biological systems under investigation. All this argues for an integrated approach to the teaching of recombinant DNA and genetic engineering in which the techniques cannot be divorced from the biology.

Nowhere are these dilemmas more painfully visible than in Alan Emery's *An Introduction to Recombinant DNA*. From the beginning Emery declares that he has not set out to write a textbook of molecular biology (of which, he adds, there are many good examples on the market). But, within a few pages, he is forced to explain DNA replication and subsequently, in the second chapter, genome organization has to be covered along with translation and transcription. The use of recombinant DNA techniques for the analysis of gene structure and function (Chapter 4) forces explanations of pseudogenes, transposons and multigene families until, at last, the author has lost the battle and the book reverts to a more integrated approach — describing various facets of recombinant DNA techniques within the context of molecular biology. Globin gene

organization, immunoglobulin genes and oncogenes are all covered in following chapters, which discuss the molecular pathology of unifactorial genetic disorders and the molecular pathology of common diseases.

By now, it is apparent that the book is severely medically orientated and it continues in this vein. *An Introduction to Recombinant DNA* might more correctly be described as a textbook on molecular biology in medicine. This is not a thorough or careful account of recombinant DNA techniques — despite their probable importance in the future, some techniques such as embryo microinjection and the use of transgenic mice receive a most cursory mention — and the application of recombinant DNA in other disciplines is confined to a single, short chapter at the end of the book.

Roger Warr's *Genetic Engineering in Higher Organisms*, a volume in Edward



Synthesis of genes from blocks of oligonucleotides. The diagram is taken from Gene Cloning: The Mechanics of DNA Manipulation by David M. Glover, published by Chapman & Hall/Methuen.

Arnold's *Studies in Biology* series, concentrates on a wide range of higher systems and thus provides a more comprehensive coverage of methods for transferring genes into animal and plant cells. It therefore might seem to complement Emery's book. However, given the limited space available to the author within the pocket-book format of this series, the treatment of the subject is necessarily light and students will need to consult some of the texts provided in the reference list for a more detailed grasp of the systems described. In the same series, Brian Clark's *The Genetic Code and Protein Biosynthesis* has been revised and updated to provide what is almost the pocket version of Adams *et al.*'s *The Biochemistry of the Nucleic Acids* — a brief but clear and very historically orientated account of the elucidation of protein biosynthesis. Initially, I rebelled against the historical treatment, but the story illustrates the intermingling of biology and techniques that is the keenest approach to molecular biology. Still, some students, begging for final answers, may

find this historical approach somewhat tortuous.

Clark resists the temptation to introduce a mandatory chapter on recombinant DNA, but no such restraint has been shown by Lin, Goldstein and Syvanen in *Bacteria, Plasmids, and Phages*. The book is subtitled (in rather large letters, I might add) *An Introduction to Molecular Biology*, something that it patently isn't. This is a general introduction to microbiology with the emphasis on bacteria and its associated plasmids and phages. Bacterial genetics and genetic control systems, plasmid genetics, phage genetics and development are covered and there are cursory descriptions of some aspects of molecular biology, for example transcription, translation and allosteric enzymes. The book is very comprehensive but not especially detailed, so, for example, the decision pathway of lambda lysogeny is covered in a couple of pages. This is a basic primer for a bacteriology/virology course, with enough basic molecular biology thrown in to prevent the student's understanding of bacterial and viral systems from breaking down. A short chapter, "DNA Restriction and Gene Cloning", cannot make up for the absence of any discussion of the use of recombinant DNA in analysing bacterial or viral systems. Once again integration may have proved the better course.

A most refreshing aspect of Rees and Sternberg's book is that it does not claim to be anything it isn't. This is almost a coffee-table account of molecular biology, containing 44 sections dealing with topics from cell structure to gene organization. The structure of various macromolecules is covered in some sections and the book finishes with sections on muscle action, nerve action and the action of hormones and antibodies. Using a large-page format, each section takes up two facing pages and includes an extensive diagrammatic explanation which is accompanied by clear, concise notes illustrating the major points. The diagrams are of superb quality, managing to convey on the two-dimensional surface of the page, the four-dimensional dynamic quality of the processes they describe. Accompanying the diagrams a carefully measured text makes this a useful volume. However, it is not a textbook to be used in isolation — it will serve its purpose best as an adjunct to other more standard and comprehensive works.

If the measure of current textbooks on molecular biology, particularly with reference to recombinant DNA, may be taken from the present crop, then with one or two exceptions they seem generally of low standard. In part this stems from the apparent desire to be topical. But topicality alone cannot serve the interests of student or teacher in molecular biology. □

S.D.M. Brown is a Lecturer in the Department of Biochemistry at St Mary's Hospital Medical School, London.

Molecular defence

Andrew McMichael

Antibodies: Their Structure and Function.

By M.W. Steward.

Chapman & Hall/Methuen: 1984. Pp. 96. Pbk £3.50, \$7.50.

Introduction to Molecular Immunology, 2nd Edn.

By Alfred Nisonoff.

Sinauer/Blackwell Scientific: 1984.

Pp. 236. Pbk \$14.95, £12.80.

Molecular Immunology: A Textbook.

Edited by M. Zouhair Atassi, Carel J. van Oss and Darryl R. Absolom.

Dekker: 1984. Pp. 725. SwFr. 129, \$47.

IN THE past few years recombinant DNA technology has made a tremendous impact on our understanding of the immune response. This revolution is still accelerating as the T cell receptor, differentiation antigens and lymphokines are cloned and sequenced. Textbooks on "molecular immunology" appearing now — and written a year or two ago — are thus in danger of appearing outdated to those whose imagination is caught by today's excitement. At the time these three books were prepared, however, the new technology had been applied to antibodies and MHC antigens. So readers should not be disappointed, because these structures lie at the heart of the discipline.

The most concise and aptly titled of the three books is Steward's *Antibodies: Their Structure and Function*. The perspective is biochemical, with a clear and detailed account of the structure of immunoglobulins. The functional properties are discussed at the level of the antibody molecules, with equal attention being given to their ability to bind antigen at one end and to trigger secondary events at the other. The nature of the antigen binding is discussed particularly well and is the book's strongest point. Less attention is given to molecular genetics, although the essential features are covered. This is a solid and well written book, suitable for undergraduate biochemists or immunologists. It should serve as a good introductory text and has a plentiful bibliography.

Nisonoff's *Introduction to Molecular Immunology* is a superb book. It concentrates mainly on immunoglobulins and takes the reader carefully through the antibody molecule, from its binding properties to primary and three-dimensional structure and on to genetic control. Starting from first principles, including a brief description of the cellular background, it should be fully comprehensible to most biologists. The clarity of the text is not the result of over-simplification and the reader is taken up to the front line in research as it was in January 1984. The illustrations are good and always help to clarify the text.

This is the second edition of an already

successful book and author and publisher are to be commended on the rapid appearance of the updated version. The new edition has been expanded by extensive rewriting of the chapter on complement and inclusion of a new chapter on the major histocompatibility complex. The latter is written with the same emphasis on biochemical and genetic information and has a brief introduction to explain why the MHC is important. This book will appeal to students at all levels, making an excellent introductory text for undergraduate courses and a useful revision text for those of us who have not followed this literature closely. I look forward to the third edition, hoping that new chapters on the T cell receptor, differentiation antigens and lymphokines will be added and explained with the same clarity.

Molecular Immunology, a multi-author work, is aimed at graduate courses and is much broader in scope than the two books discussed above. Unfortunately it seems to have taken longer to appear and recent advances make it look somewhat dated. The best parts of the book are the extensive (139-page) discussion of antigens and the following section on antibodies. The discussion of antigens is made up of several

interesting chapters, although some have a rather narrow perspective — for instance, Atassi exclusively describes his own work on defining antigenic epitopes on myoglobin, lysozyme and haemoglobin — while the section on antibodies is less detailed than that found in the other two books. Fearon's chapter on complement, however, is one of the best short descriptions of this topic that I have seen.

The section on cellular immunology lacks molecular information and may seem to overemphasize phenomenology, but it should be realized that most of the new discoveries are confirming and explaining the observations of traditional immunology. There are several chapters on methodology, with a particularly good section on electron microscopy by M.A. Gonda (though I was surprised there was only a brief description of flow cytometry in the account of cell separation techniques). The balance of this book is rather different to the other texts, and it should therefore form a useful addition to library collections. □

Andrew McMichael is a Professor in the Nuffield Department of Clinical Medicine at the John Radcliffe Hospital, Oxford.

Tackling immunity

Brian Solomon

Essentials of Immunology.

By W.H. Hildemann.

Elsevier: 1984. Pp. 146. Dfl. 60, \$19.50.

Immunology: An Introduction.

By Ian R. Tizard.

Holt Saunders/Saunders College: 1984.

Hbk £21, \$31.95; pbk £7.95.

CHOOSING an original title for a book on basic immunology must nowadays be a thankless task. Unfortunately, *Essentials of Immunology* is too similar to the title of the well-established *Essential Immunology*, by Ivan Roitt, which is very different in style. In his preface the late Bill Hildemann suggested an alternative title of *Immunology in a Nutshell* and after reading the book I consider that this would have been much more appropriate.

The book's stated origins lie in a lecture course for medical and science students in the health sciences. This probably explains why the text resembles lecture notes and there is a heavy reliance on diagrams and complex tables. There are some paragraphs where there is a true flow of words, but usually facts are given in a jerky note form and the reader is soon referred to a table or figure for more information. With such a sparse text, generalizations have had to be made, inevitably so, but some are dangerously misleading. For example, the process of opsonization is not "complement-dependent" (for IgG) but IgM will function as an opsonin when bound

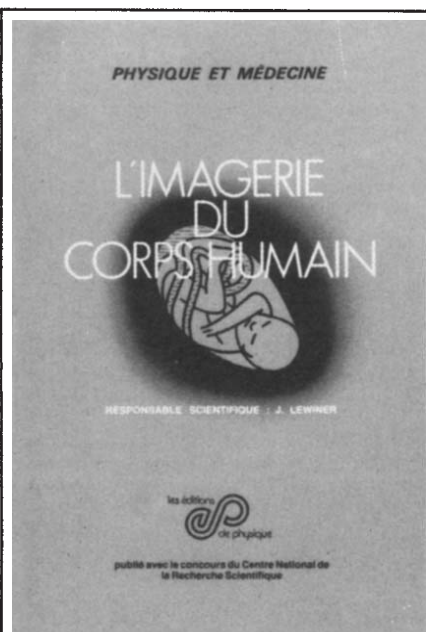
with C1q; the Chediak-Higashi syndrome is chiefly notable for a deficiency of natural killer cells; and the propensity for immature B cells to be driven into tolerance by antigen (a strong teaching point) is not even mentioned.

Although the book concentrates on human immunology there are sudden switches to mouse or chicken experiments without warning. This occurs particularly in the "Overviews" at the end of each chapter. For example, the statement "that fetal and newborn mammals acquire passive immunity by placental passage of IgG and by assimilation of sIgM and sIgA from the colostrum" is bound to confuse medical students.

As a cellular immunologist, the author regards immunoglobulins as "ancillary to cell-mediated immune functions", and the sheer magic of the widely different biological functions of the various immunoglobulin classes is lost in a massive table. There is only one paragraph on ontogeny, while considering Bill Hildemann's enormous contribution to research on immunorecognition in invertebrates it was also disappointing to find only two short paragraphs on phylogeny with one unnecessary table and another with misleading information. In short, I found too little nut inside the nutshell.

In Ian Tizard's *Immunology: An Introduction*, the reader is struck at once by the confidence of the author's writing. Many grey areas of my knowledge were dispersed by this book. At the same time, newcomers to immunology will find all their basic requirements for getting to grips with the subject. The opening chapters follow a

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Le médecin utilise de très nombreux outils nouveaux pour l'imagerie du corps humain, la thérapie, la surveillance et la régulation des paramètres physiologiques. L'interaction est donc de plus en plus forte entre médecins et physiciens.

Consacrés aux méthodes d'imagerie, les divers chapitres de cet ouvrage décrivent chacun une technique particulière et ont été écrits par des spécialistes. Ils concernent des domaines déjà bien utilisés tels que l'imagerie acoustique ou encore très futuristes comme la radiographie par diffusion nucléaire. On trouvera ainsi successivement les chapitres consacrés à :

- l'imagerie acoustique,
- l'imagerie micro-onde,
- la thermographie micro-onde,
- la tomographie d'émission,
- la radiographie par diffusion nucléaire.

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conventional pathway through an early history of vaccines (Pasteur, Jenner) to antigens, major histocompatibility complex and phagocytes, followed by inflammation. Next, we suddenly leave cells for three chapters on antibodies before "tissues of the immune system" are approached. I feel a more logical order would have been to describe lymphoid organs and the differentiation of lymphocytes, then go on to discuss the induction of an immune response which would lead to a description of end products such as lymphokines and antibodies.

Among the antibodies much attention is given to allotypes, to the neglect of the sugars. Immunoglobulins are glycoproteins, and although the role of the oligosaccharides is still largely unknown they may help to dictate the various biological functions of the immunoglobulins. To indicate in a figure that IgE only has sugars bound to the C_H2 domain (like IgG) is incorrect as six oligosaccharides have been reported on three different C_H domains of IgE. There follows an excellent chapter on how to measure antigen-antibody reactions and antibody affinity, including a lucid section on antigen-antibody interaction with Fc receptors on cells.

As the author leads us into the jungle of immunoregulatory processes, he looks back at us over his shoulder to offer encouragement to "who has managed to follow so far"; we later graduate to a reader "who has managed to follow so far"! This is not because the book is difficult to understand, rather a sympathetic way of apologizing for the complexity of the subject. A stimulating chapter "Immunity at Body Surfaces" precedes another on vaccines which are dealt with very competently. However, absent here is an assessment of how successful vaccines have been in the improvements in public health from the turn of the century to the present day, and in the introduction of antibiotics. As vaccines are designed to induce memory cells and many properties of murine B memory cells have been described it was a pity not to include them.

The highlight and declared *modus operandi* of the book lies in the account of mechanisms of resistance to disease. This topic is bravely treated by separately dealing with bacteria, viruses, protozoa and helminths, an approach to a difficult subject which exposes many of the "nasties" practising immune evasion. A certain weakness in defining tropism with the microorganism's reliance on specific receptors on target cells can be excused as being "non-immunological", but I missed any account of the Duffy blood group - *Plasmodium vivax* story. Still, I like this book — it is how I would have described immunology myself and it has something in it for everyone. □

Brian Solomon is a Lecturer in the Department of Bacteriology at the University of Aberdeen.

Enzymology in the
golden ages

Keith Tipton

Enzyme Structure and Mechanism, 2nd
Edn.

By Alan Fersht.

W.H. Freeman: 1985. Pp.475. Hbk
\$24.95, £28.95; pbk £14.95.

ALTHOUGH an understanding of the behaviour of enzymes is essential for any full appreciation of the behaviour of living systems at the biochemical level, enzymology is too often regarded as being an esoteric and somewhat purposeless pursuit or a pedestrian and ossified technology. Anyone with such views should read this book. It is the author's contention that we are now entering a new golden age of enzymology, but this scholarly and enthusiastically written account suggests that such an age has existed for many years and will continue for many more.

A book of this size could not be expected to provide a comprehensive coverage of all aspects of enzymology and it is particularly strong in areas such as transient kinetics, enzyme mechanisms, and specificity and editing mechanisms, in which the author has been particularly active himself. However the generally well-chosen reference lists should allow readers to develop their knowledge in areas that receive less detailed coverage, for example steady-state kinetic analysis and the behaviour of metabolic pathways. The book is never dull and in controversial areas, such as the evolution of enzyme kinetic power, the author presents his own arguments cogently and convincingly.

Many will be familiar with the first edition of this work. In addition to the revision of much of the original material there are several new chapters, among them an account of enzyme stereospecificity, a topic which is often neglected in books of this type, and an excellent treatment of genetic engineering and enzymology. This latter chapter includes a particularly valuable consideration of the techniques of site-specific mutagenesis and their importance to the future development of the study of enzymes.

I have been recommending the first edition of this book to students, both undergraduate and graduate, since its publication in 1977. The appearance of this revised and expanded version is most welcome. I recommend it most highly as a stimulating and informative guide in which the ideas and principles behind our current understanding of enzymology are presented in such a way as to capture much of the excitement of the subject. □

Keith Tipton is Professor and Head of the Department of Biochemistry at Trinity College, Dublin.

Modern microbes

G.P.C. Salmond &
R. Whittenbury

Biology of Microorganisms, 4th Edn.

By Thomas D. Brock, David W. Smith and Michael T. Madigan.

Prentice-Hall: 1984. Pp.847. Hbk \$51.25, £46.70; pbk \$26.95, £15.95.

Microbiology, 4th Edn.

By George A. Wistreich and Max D. Lechtman.

Macmillan, New York*/Collier Macmillan, London: 1984. Pp.909. £40.

"MAINLY old wine in new(ish) bottles" describes, perhaps unkindly, the two textbooks reviewed here. The first, a new edition of *Biology of Microorganisms*, has emerged with two new co-authors joining the originator of the enterprise, T.D. Brock. The product is a success, building on the previous tried and proved formula. Updating, particularly of the genetics and genetic engineering contributions is the major change from previous editions. Brock, the senior author, is widely regarded as the working general microbiologist's general microbiologist; consequently the content, style and depth of treatment, choice of illustrations, and table and diagram construction fit well with a general microbiologist's conception of what constitutes a good student textbook.

The microbe in its own right holds sway here, we are please to observe; for instance, accounts of structure, function, biochemistry and genetics, and of the microbe in relation to the environment, as a parasite and as a symbiont, all hang together in a way which draws the reader fairly painlessly into the complexity of modern microbiology. In all there are 19 chapters, with useful appendixes, a glossary and a reasonably comprehensive index. A particularly useful chapter is the last one, which describes representative prokaryotic groups and is, in effect, a series of compact essays conveying the essential biology and the unique attributes of each group. This book compares well with the other leaders in the field and is a strong contender for adoption by undergraduates reading courses in which microbiology is a significant component.

Wistreich and Lechtman's *Microbiology*, also in its fourth edition, represents a brave but, in our eyes, unsuccessful attempt to produce a modern balanced textbook of general microbiology. The book is organized into eight parts; the relative emphasis on the constituent subjects, however, is very uneven. For example Part II is designed to inform on the wide topics of growth, cultivation, metabolism and genetics. This covers only three chapters (6, 7 and 8) ac-

counting for about 75 pages. On the other hand, medical microbiology and immunology start at Chapter 19 and go through to the end of Chapter 28, taking approximately half of the book. The rest of the book covers various matters, including microbial ecology, industrial microbiology, cell structure and function, and virology.

Perhaps worse than the imbalance in treatment is that the text is superficial in terms of content. Indeed, many parts of the book are likely to be totally misleading, either due to bad grammar or errors of fact. Chapter 8 on microbial genetics is a particularly illuminating example on both counts. It is littered with confusing information, contains mistakes and is badly out of date (for instance, no mention is made of yeast genetics). The poor balance of the book shows up even here where only four

pages are dedicated to the topic of genetic manipulation — probably the cornerstone technique of modern biology!

There are some good points to the book, however. On the whole, it is easy to read, there is a wealth of diagrams in both black-and-white and colour, and the photographs are of a high standard. Each chapter begins by listing the things that you should know, and ends (in a form suspiciously like a list of examination questions) with a summary of the salient points plus questions and suggestions for further reading. Our impression, then, is that although this is an excellent microbiology "picture book" the very heavy emphasis on things medical severely perturbs its balance. □

G.P.C. Salmond is a Lecturer and R. Whittenbury a Professor in the Department of Biological Sciences at the University of Warwick.

Bodily functions

Ole H. Petersen

Anatomy and Physiology, Vol.1, 2nd Edn.

By Edwin B. Steen and Ashley Montagu.

Barnes and Noble/Harper & Row: 1984. Pp.406. Pbk \$6.95, £4.95.

Essentials of Human Anatomy and Physiology.

By Elaine Nicpon Marieb.

Addison-Wesley: 1984. Pp.355. Pbk \$17.95, £18.

Physiology of the Human Body, 6th Edn.

By Arthur C. Guyton.

Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.691. Hbk £32.50, \$37.95; pbk £8.50.

Essentials of Physiology, 2nd Edn.

By J.F. Lamb, C.G. Ingram, I.A.

Johnston and R.M. Pitman.

Blackwell Scientific/C.V. Mosby: 1984. Pp.465. Hbk \$18.95; pbk £12.50.

IN THE preface to Guyton's *Physiology of the Human Body* it is correctly stated that "... physiology continues in a dynamic stage of discovery with new knowledge of basic physiological concepts generated each day". Unfortunately, these new concepts do not figure prominently in any of the four textbooks reviewed here, and this is clearly due to the practice admitted in the preface to Lamb *et al.*'s *Essentials of Physiology*: "The reference list included is generally that which we found useful and usually consists of other secondary references, i.e. larger texts, monographs, etc". In other words, authors of physiology textbooks mostly get their information from other textbooks of physiology.

Steen and Montagu's *Anatomy and Physiology* is not a real textbook, but more a compendium of basic anatomical and physiological facts presented in a small-format paperback. It is in two volumes, but at present only Vol. 1 covering blood, cir-

culatation and the muscular and digestive systems is available. The index is not bad and when the companion volume appears this work could well be useful for last-minute revision purposes.

Essentials of Human Anatomy and Physiology is designed to meet the needs of students in the allied health fields and as such is in direct competition with the more attractive *Human Anatomy and Physiology* by Solomon and Davis which was reviewed last year (*Nature* 308, 129; 1984). The anatomy sections are better than those dealing with physiology, in which basic errors can be found; for example the figure illustrating exchange of oxygen and carbon dioxide between tissues and capillaries indicates wrongly that the red cells are only concerned with oxygen and that all carbon dioxide is transported in the plasma.

Guyton's *Physiology of the Human Body*, now appearing in its sixth edition, is a thoroughly professional book with particularly good black-and-red diagrams and a text which is easy to read. It covers the same ground as *Textbook of Medical Physiology* by the same author, but with less emphasis on medical aspects. Each chapter starts with a brief overview summarizing the most important points and ends with a short list of questions and references. However, in spite of the addition of some new references, the book could essentially have been written 20 years ago. Key topics such as control of the heart, nerve action potential and transport in the kidney tubules are discussed without any reference to the important results obtained with the new methodologies of recent years. This can make the presentation unnecessarily complicated, when, for example, the electric action potential is explained in terms of ill-defined permeability changes instead of through opening and closure of specific ion channel molecules in the plasma membrane.

Unfortunately, Lamb *et al.*'s *Essentials of Physiology*, which is aimed at medical,

*See footnote p.40

dental and science students, is only marginally better than Guyton's book in explaining the basic bioelectric phenomena which are central to so many physiological processes. Indeed one crucial figure (2.3 on p.29) is both confusing and incorrect by mixing up permeability and conductance, and the muddle is complete when megaohm is used as the unit! *Essentials of Physiology* is much more up to date than Guyton's textbook, but for a second edition it contains an astonishing number of omissions, mistakes and inconsistencies. Lundsgaard, my teacher as an undergraduate student in Copenhagen, often expressed the view that those unable to describe the effects of stimulating the sympathetic or parasympathetic nerves to the heart should not expect to survive his examination, but a present-day student relying on *Essentials of Physiology* would be in trouble as the positive inotropic action of sympathetic nerve stimulation is not mentioned explicitly in the section on the regulation of cardiac output. How marvellous if this and the other textbooks were to explain that this inotropic effect is due, at least in part, to an increased flow of calcium ions into the myocardial cells resulting from activation of selective calcium channels in the plasma membrane by the elevated intracellular cyclic adenosine monophosphate concentration (Reuter's excellent review of the process — *Nature* 301, 569–574; 1983 — is recommended to all those writing a physiology textbook).

Lamb's book nevertheless has many positive features, such as the introductory ideas in the first chapter with its instructive and amusing figures, the excellent description of the mechanics of muscle contraction and the useful appendix on units and formulae. But one cannot help feeling that the authors have failed to read carefully and critically the whole text before sending it to the printers; thus the hormone cholecystokinin is variously referred to as CPZ, pancreozymin, CCK-PK, CCK and CCK-PZ, cyclic adenosine monophosphate is introduced as cyclic AMP without explaining the abbreviation and some of the diagrams (for example that of the exocrine pancreas on p. 155) are lacking in precision and likely to be misunderstood by many students.

None of these four textbooks provide good examples of how physiological data obtained at the cellular level can help us to understand important disease symptoms. The osmotic diuresis seen in diabetes mellitus, for example, can be simply and logically explained on the basis of the known transport characteristics of the different segments of the nephron, but none of the authors even mention the term osmotic diuresis. It is indeed a pity that many such good opportunities for helping students to use physiological reasoning have been missed. □

Ole H. Petersen is George Holt Professor of Physiology at the University of Liverpool.

The developing side of biology

Mary Bownes

Developmental Biology, 2nd Edn.

By Leon W. Browder.

Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.748. Hbk £32.95, \$37.95; pbk £17.95.

Developmental Control in Animals and Plants, 2nd Edn.

Edited by C.F. Graham and P.F. Wareing.

Blackwell Scientific: 1984. Pp.519. Hbk \$37; pbk £18.50.

Genetics and Development.

By James H. Sang.

Longman: 1984. Pp.398. Pbk £13.95, \$25.

THE rapid advances in our understanding of the genetic control of development and the molecular basis of developmental mechanisms make this an exciting period for students of developmental biology. All three of the books reviewed here cover both plants and animals, which is a major step forward, although in each case the space devoted to plant development is rather less than that devoted to animals.

Browder's *Developmental Biology*, now in its second edition, is the most basic text of the three and is extremely well written, up to date and enjoyable to read. The introduction provides a historical look at the topic, sets the context for the following five sections and briefly describes the main aspects of animal development. The subsequent sections each contain two to four chapters, advanced topics being dealt with in essays set aside from the main body of text. "The Cell in Development" (Section 2) covers not only the basic concepts of determination and differentiation but also chromatin and its structure, RNA and protein synthesis and the mechanisms of regulating gene expression, both transcriptional and post-transcriptional, and finally the cytoskeleton. This should give students a good idea of where many of the advances are being made, and the author provides sufficient background in molecular biology and genetics to make the section genuinely valuable rather than merely superficial.

There are detailed analyses of spermatogenesis, oogenesis and plant gametogenesis, followed by chapters on fertilization and "becoming multicellular". There is then a return from the more cellular experiments to the molecular, and the role of gene expression during the initiation of development is well described. The final section describes the processes of forming a multi-layered embryo, and generating the wide range of cell types organized such that they create a functional organism. Throughout, the experimental basis of the subject is emphasized and well explained, and the

text is beautifully illustrated with many clear and informative photographs and diagrams. This is a book that all students interested in development will find very rewarding.

The other two books are aimed at advanced undergraduates and, to some extent, at postgraduates and research workers too. Graham and Wareing's *Developmental Control in Animals and Plants*, also in its second edition, is a multi-author effort. The disadvantages of such volumes are well-known — lack of continuity of style and approach — yet, of course, in compensation each person is an expert in a given area. My expectation was that the book would thus be well up to date, but although the chapters are generally well written and interesting, only the final contribution by Woodland and Old ("Gene Expression in Animal Development") includes the kind of very recent experimental analysis one would hope for. This is fortunate since this area is moving especially fast.

The six parts cover developmental processes, the origin and maintenance of cell heterogeneity, cell communication, pattern and form, hormones and the molecular biology of development. One of the main aims of the authors was to present both plant and animal development at an advanced level. Several chapters in Part 5, "Hormones in Development", fall rather short of this. In the plant chapters there is no mention of the current controversy over plant growth factors, the authors taking a very classical approach to the topic which is perhaps no longer appropriate, while the chapter on hormones in animal development is somewhat narrow, concentrating rather too much on steroid hormones and the chick oestrogen and *Xenopus* vitellogenin systems.

Sang's *Genetics and Development*, intended for those interested in the genetic mechanisms controlling development, fills a long-standing gap in the textbook market. It is a stimulating and detailed account of the current state of a rapidly moving area of research, aiming to provide the basis for students to be able to follow research papers. This it certainly does.

All of the six sections are heavily biased towards *Drosophila*, but this does not prevent the author meeting his objectives. The section on genome organization covers details of sequence organization and chromatin structure, polytene chromosomes and various models for regulating gene expression. Included under "Mutations" are definitions of what is meant by dominance, dosage compensation and complementation, analysis of complex loci and discussion of how the study of mutations enabled the structure of genes to be unravelled. Hormonally controlled and developmentally regulated genes are described and, finally, mutations which cause lethality at various developmental stages are analysed. In a section on tissue-determination and regeneration the

author concentrates on the production of mosaics and chimaeras to analyse these problems. A special account is devoted to the very topical area of determination mutants where the homoeotic mutants are investigated along with the problems of sex determination; this is particularly useful as many of the molecular studies on these systems demand an understanding of large amounts of background genetics which are rarely documented in such a complete form that students can see how progress has been possible and begin to tackle reading the research papers. The final section on future developments concentrates on cellular

systems, a personal view of which areas of research are likely to be fruitful, and the book ends with an analysis of problems still to be solved.

In this book Sang has succeeded in bringing together the classical genetic approach of using mutations to study development with the more recent molecular techniques. I'm sure it will be of great use to students of both genetics and developmental biology. □

Mary Bownes is a Lecturer in the Department of Molecular Biology at the University of Edinburgh.

Nervous connections

Julian Lewis

Development of Nerve Cells and Their Connections.

By W.G. Hopkins and M.C. Brown.
Cambridge University Press: 1984.

Pp. 137. Hbk £16, \$29.95; pbk £6.95, \$13.95.

Principles of Neural Development.

By Dale Purves and Jeff W. Lichtman.
Sinauer/Blackwell Scientific: 1985.

Pp. 433. \$32.50, £29.50.

From Neuron to Brain, 2nd Edn.

By Stephen W. Kuffler, John G. Nicholls and A. Robert Martin.

Sinauer/Blackwell Scientific: 1984.

Pp. 651. Hbk \$30; pbk £14.80.

THE development of the nervous system is not yet one of those subjects where a set of fundamental principles emerges neatly from a series of crucial experiments. Perhaps it will be one day: therein lies the challenge. To optimists, the published literature seems like the jumbled pieces of a giant jigsaw puzzle: find the right arrangement, and a beautifully coherent picture will emerge. Others, with less faith in universal principles, may perceive only a rambling mass of natural history anecdotes, fascinating to relate, but with no more logical unity than the Arabian Nights entertainments.

In *Development of Nerve Cells and Their Connections*, Hopkins and Brown adopt a sober and businesslike approach: their short, well-referenced and very reasonably-priced book offers a terse, dispassionate review of what is known about neural development, beginning with the genesis of neurones in the embryo and ending with plasticity and regeneration in the adult. They do not attempt to dramatize the data, and the reader is often left to supply his own emphases. For this reason, and because the presentation is rather condensed and bare of practical details or pictures of the numerous experimental systems discussed, the book, and especially its first half, may be hard going for newcomers to the subject. I found the second half, dealing with the modification

of neural connections, the more valuable. As one might expect from these authors, it includes a particularly lucid discussion of synapse elimination and of nerve sprouting. And it is the only one of the books under review that has the courage to tackle head-on, in a few useful and well-organized pages, the problem of learning and memory; the other two venture only a sideways glance at this central enigma.

Purves and Lichtman's larger and more ambitious book, *Principles of Neural Development*, is a masterpiece: comprehensive but not stodgy, clear, accurate, elegantly written, beautifully and copiously illustrated. Even if you don't like words, you will be seduced by the pictures. While it does not offer any revolutionary new synthesis, the book succeeds, better than any previous text on the subject, in the very difficult task of weaving the inchoate mass of data on neural development into a single coherent narrative. Despite the 47 pages of references, and a scholarly care in assigning credit and priorities, Purves and Lichtman avoid the dismal blight that often afflicts *Annual Reviews*, where so many experiments are squeezed into so small a space that no life is left in them. Key experiments are made vivid and concrete by means of figures displaying the methods, materials and observations, reproduced as far as possible from the original papers.

The historical antecedents of modern work are particularly well portrayed. I enjoyed especially the short (one page) biographies of scientists, past and present, who have played major parts in the story. These sketches provide more than the usual bland catalogue of dates and discoveries, managing, in a few paragraphs, not only to explain how and why the scientific achievements were important, but also to bring the characters to life. Purves and Lichtman also have an excellent way with unresolved scientific conflicts: they set out the arguments fairly and squarely, and then tactfully and undogmatically offer their own judgement. As from all the best books, one learns about a great deal more than the title promises, from the principles of Nomarski interference contrast to the discomforts of wearing a reversing pseudophone.

From Neuron to Brain, by Kuffler, Nicholls and Martin, is the second edition of a classic. The first edition was for me and many others a memorable and engrossing introduction to the cellular basis of neurophysiology. It succeeded in large measure by sticking to the excellent principle that it is better — and more fun — to learn ten things well than a hundred things superficially. The authors focused on topics that they knew intimately, and conveyed their fascination with them.

The new edition retains the structure and much of the text of the old, but is enlarged by about 30%. It opens, as before, with a discussion of how neurones represent information about the visual world, updated where appropriate but as good as ever. Spurred on and sustained by the sense that the workings of neurones are the key to the higher functions of the brain, the reader is then taken through the more arduous analysis of the electrical properties of neurones and of synaptic transmission.



Konrad Lorenz with his geese. The picture is reproduced from one of the short biographies in *Principles of Neural Development*.

The revision here has been more drastic, making the account more rigorous, more complete and less readable for beginners. New topics such as patch clamp recording of currents through single channels are covered clearly and concisely, though sometimes without as much adjustment of the balance of emphasis as they deserve. One does not get the sense that any of the new techniques and discoveries of the past eight years have yielded any radical changes in our perception of neurones or the nervous system, or made any significant part of the material in the first edition obsolete. Is it really true, as stated in the "Concluding Remarks", that "we still do not understand the basic mechanisms that underlie changes in membrane permeability and active transport"? □

Subsequent chapters include, together with the old material, many good new things, from sensitization in *Aplysia* to the role of the basal lamina in synapse formation, and a whole new chapter on the control of movement. This is still an outstanding book.

Julian Lewis is a Lecturer in the Department of Anatomy at King's College, University of London.

Index to textbooks reviewed

Advanced Organic Chemistry, Part A, 2nd Edn: Structure and Mechanisms (by F.A. Carey and R.J. Sundberg)	30
Advanced Plant Physiology (M.B. Wilkins, ed.)	42
Anatomy and Physiology, Vol. 1, 2nd Edn (by E.B. Steen and A. Montagu)	49
Animal Behavior: An Evolutionary Approach, 3rd Edn (by J. Alcock)	36
Antibodies: Their Structure and Function (by M.W. Steward)	47
Aspects of a Stratigraphic System: The Devonian (by D.L. Dineley)	27
Astrophysical Techniques (by C.R. Kitchin)	25
Astrophysics (by R. Bowers and T. Deeming)	24
Atlas of Invertebrate Macrofossils (J.W. Murray, ed.)	29
Bacteria, Plasmids, and Phages: An Introduction to Molecular Biology (by E.C.C. Lin <i>et al.</i>)	46
Basic Marine Biology (by A.A. Fincham)	38
Behavioural Ecology: An Evolutionary Approach, 2nd Edn (J.R. Krebs and N.B. Davies, eds)	37
Biogeography: An Ecological and Evolutionary Approach, 4th Edn (by C.B. Cox and P.D. Moore)	40
Biological Oceanographic Processes, 3rd Edn (by T.R. Parsons <i>et al.</i>)	38
Biology of Animal Behavior (by J.W. Grier)	36
Biology: An Introduction (by K.D. Johnson <i>et al.</i>)	33
Biology of Microorganisms, 4th Edn (by T.D. Brock <i>et al.</i>)	49
Biology: The Unity and Diversity of Life, 3rd Edn (by C. Starr and R. Taggart)	33
Bioscope, 2nd Edn (by T.A. Easton and C.E. Rischer)	33
Cell Biology, 2nd Edn (by G. Karp)	45
Cells and Organelles, 3rd Edn (by E. Holtzman and A.B. Novikoff)	45
Chemistry of the Elements (by N.N. Greenwood and A. Earnshaw)	30
Chloroplast Metabolism: The Structure and Function of Chloroplasts in Green Leaf Cells, revised edn (by B. Halliwell)	42
Chloroplasts (by J.K. Hooper)	42
Class Experiments in Plant Physiology (by H. Meidner)	42
Classical Mechanics (by B.P. Cowan)	23
Community Structure and the Niche (by P.S. Giller)	39
Concepts of Ecology, 3rd Edn (by E.J. Kormondy)	37
Development of Nerve Cells and Their Connections (by W.G. Hopkins	

Textbook supplement — prices

Where possible both dollar prices in the United States and sterling prices in Britain are given in the bibliographical details for each book. Export prices will generally be higher. If a dollar or sterling price is not cited, the book is not available in one of the two countries. However readers should check both price and availability of books before ordering.

and M.C. Brown)	51
Developmental Biology, 2nd Edn (by L.W. Browder)	50
Developmental Control in Animals and Plants, 2nd Edn (C.F. Graham and P.F. Wareing, eds)	50
Ecology: Principles and Practice (by W.H. Dowdeswell)	37
Environmental Systems: An Introductory Text (by I.D. White <i>et al.</i>)	26
Enzyme Structure and Mechanism, 2nd Edn (by A. Fersht)	48
Essentials of Bio-organic Chemistry (by R.W. Hanson)	31
Essentials of Human Anatomy and Physiology (by E.N. Marieb)	49
Essentials of Immunology (by W.H. Hildemann)	47
Essentials of Physiology, 2nd Edn (by J.F. Lamb <i>et al.</i>)	49
Evolution: A Modern Synthesis (by W.H. Dowdeswell)	34
The Evolving Continents, 2nd Edn (by B.F. Windley)	27
Exploring Biology, 2nd Edn (by P.S. Camp and K. Arms)	33
A First Course in General Relativity (by B. Schutz)	22
From Cells to Atoms: An Illustrated Introduction to Molecular Biology (by A.R. Rees and M.J.E. Sternberg)	46
From Darwin to Behaviourism: Psychology and the Minds of Animals (by R. Boakes)	35
From Neuron to Brain, 2nd Edn (by S.W. Kuffler <i>et al.</i>)	51
Fundamentals of Air Pollution, 2nd Edn (by A.C. Stern <i>et al.</i>)	28
General Relativity (by R.M. Wald)	22
General Relativity and Relativistic Astrophysics (by N. Straumann)	22
General Zoology, 6th Edn (by C.A. Villee <i>et al.</i>)	32
Genesis on Planet Earth: The Search for Life's Beginning, 2nd Edn (by W. Day)	43
The Genetic Code and Protein Biosynthesis, 2nd Edn (by B.F.C. Clark)	46
Genetic Engineering in Higher Organisms (by J.R. Carr)	46
Genetics, 2nd Edn (by C.J. Avers)	44
Genetics, 3rd Edn (by U. Goodenough)	44
Genetics and Development (by J.H. Sang)	50
Geomorphology (by R.J. Chorley <i>et al.</i>)	26
The History of the Earth's Crust (by D.L. Eicher <i>et al.</i>)	27
Human Genetics (by E.A. Carlson)	44
Immunology: An Introduction (by I.R. Tizard)	47
Inorganic Chemistry: Principles of Structure and Reactivity, 3rd Edn (by J.E. Huheey)	30
Insect Biology: A Textbook of Entomology (by H.E. Evans)	32
Insect Physiology, 8th Edn (by V.B. Wigglesworth)	32
Integrated Principles of Zoology, 7th Edn (by C.P. Hickman <i>et al.</i>)	32
Introduction to the Algae, 2nd Edn (by H.C. Bold and M.J. Wynne)	41
Introduction to Molecular Immunology, 2nd Edn (by A. Nisonoff)	47
An Introduction to Recombinant DNA (by A.E.H. Emery)	46
Marine Biology (by H.V. Thurman and H.H. Webber)	38
Marine Ecological Processes (by I. Valiela)	38
Membranes and their Cellular Functions, 3rd Edn (by J.B. Finean <i>et al.</i>)	43
Microbiology, 4th Edn (by G.A. Wistreich and M.D. Lechtman)	49
Molecular Immunology: A Textbook (by M.Z. Atassi <i>et al.</i>)	47
The Nature of the Environment: An Advanced Physical Geography (by A. Goudie)	26
Neuroethology (by J.M. Camhi)	36
Organic Chemistry (by G.M. Loudon)	30
Patterns of Human Heredity: An Introduction to Human Genetics (by J.R. Brennan)	44
Physics of Amorphous Materials (by S.R. Elliott)	26
Physics of Planetary Interiors (by G.H.A. Cole)	25
The Physiology of Flowering Plants: Their Growth and Development, 3rd Edn (by H.E. Street and H. Öpik)	42
Physiology of the Human Body, 6th Edn (by A.C. Guyton)	49
Planets and Their Atmospheres: Origin and Evolution (by J.S. Lewis and R.G. Prinn)	24
Plant Molecular Biology (by D. Grierson and S. Covey)	41
Pollution of our Atmosphere (by B. Henderson-Sellers)	28
Population Biology: The Coevolution of Population Dynamics and Behavior (by J.M. Emlen)	40
Predation (by R.H. Taylor)	39
Prehistoric Europe (by T. Champion <i>et al.</i>)	28
Primate Behaviour and Social Ecology (by H.O. Box)	34
Principles of Ecology (by R.J. Putman and S.D. Wratten)	37
Principles of Neural Development (by D. Purves and J.W. Lichtman)	51
Psychological Testing (by J.R. Graham and R.S. Lilly)	35
The Solar System (by B.W. Jones)	24
Solid State Chemistry and its Applications (by A.R. West)	29
Spectral Problems in Organic Chemistry (by R. Davis and C.H.J. Wells)	30
Structural Inorganic Chemistry, 5th Edn (by A.F. Wells)	30
The Techniques in Genetic Engineering Video Library (R. Williamson, series ed.)	45
Themes in Biogeography (J.A. Taylor, ed.)	40
Theoretical Concepts in Physics (by M.S. Longair)	23
The Third Dimension in Organic Chemistry (by A. Bassindale)	30
The Universe of Galaxies: Readings from Scientific American (compiled by P.W. Hodge)	24
Vertebrate Natural History (by M.F. Willson)	34
Vertebrate Zoogeography and Evolution in Australasia (M. Archer and G. Clayton, eds)	39
A Workbook for Astronomy (by J. Waxman)	24

Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens

Ten Feizi

Applied Immunochemistry Research Group, Division of Communicable Diseases,
Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK

The hope that hybridoma antibodies would reveal unique cell surface antigens during embryogenesis, differentiation and oncogenesis has been replaced by the realization that such antigens are mainly carbohydrate structures of glycoproteins and glycolipids occurring in many cell types. These findings either may reflect limitations in the methods of selection of hybridoma antibodies or may point to important roles for the diverse carbohydrate structures as receptors for regulators of cell growth and differentiation.

MONOCLONAL antibodies of desired specificity can be produced by the hybridoma technique¹, providing a rapid means of detecting antigens which change during cellular differentiation and maturation (differentiation antigens)². The feasibility of this approach was apparent when a hybridoma antibody of this type was produced incidentally, following immunization of a rat with mouse splenocytes³. Serological studies showed that this antibody (M1:22:25:8) which recognizes an antigen with stage-specific expression in the early mouse embryo, becoming restricted only to certain cells in the adult mouse, has Forssman-type specificity³. Subsequent biochemical studies⁴ confirmed that the antibody recognizes the carbohydrate sequence of the Forssman antigen (structure 20, Table 2), found commonly on glycolipids⁵ and occurring also on glycoproteins⁶.

Developmental biologists were among the first to use hybridomas to generate 'tailor-made' antibodies to surface antigens of embryonic cells⁷. The antigens, however, seemed elusive; they could not be identified readily by the usual techniques of immune precipitation and polyacrylamide gel electrophoresis. Several of them, including some oncofetal (tumour-associated) antigens, have been shown subsequently to be either carbohydrate sequences on high relative molecular mass (M_r) glycoproteins (or polysaccharides) which cannot be radioiodinated or metabolically labelled with ³⁵S-methionine to sufficiently high specific activities and do not penetrate readily the polyacrylamide gels⁸, or they are associated with glycolipids⁹. I review here antigens that have been singled out by monoclonal antibodies as differentiation or tumour-associated antigens and have been shown later to be specific carbohydrate sequences. I discuss their possible roles and conclude by citing some examples of carbohydrate sequences to which biological roles have been assigned without immunological techniques.

Natural monoclonal antibodies

Developmentally regulated antigens were first identified as carbohydrate structures by experiments with certain natural monoclonal antibodies, anti-I and anti-i, occurring in the sera of patients with an autoimmune haemolytic disorder (reviewed in ref. 10) and recognizing two developmentally regulated antigens, I and i, of human erythrocytes. The i antigen is a prominent erythrocyte antigen of the human fetus, diminishing after birth and during the first year of life, while the related antigen, I, increases to the adult concentration¹¹. These antigens are now known to be carbohydrate structures on glycoproteins and glycolipids of erythrocytes and other cell types¹²⁻¹⁴, consisting of repeating units of *N*-acetylglucosamine (Gal β 1 $\rightarrow$ 4GlcNAc). This disaccharide sequence is also termed the Type 2 blood group chain¹⁵ or backbone structure¹⁶, to distinguish it from the isomer Gal β 1 $\rightarrow$ 3GlcNAc (Type 1); both sequences may serve as precursor chains, converted into blood group antigens by

further glycosylations. Unbranched oligosaccharides of three or four repeating *N*-acetylglucosamine units joined to each other by a β 1 $\rightarrow$ 3 linkage (see structure 1, Table 1) express the i antigen^{17,18}, whereas the corresponding branched structures, formed by β 1 $\rightarrow$ 6 linkage of *N*-acetylglucosamine units to internal galactose residues of the repeating sequence (as in structure 2, Table 1), express the I antigen^{19,20}. During the first year of life, there may be an increased activity of a glycosyltransferase (branching enzyme)^{21,101} which transfers *N*-acetylglucosamine via a 1 $\rightarrow$ 6 linkage to a previously formed linear chain.

Because undifferentiated cells of the early mouse embryo contain surface glycoproteins with poly-*N*-acetylglucosamine sequences²², we used monoclonal anti-I and anti-i antibodies against different epitopes on the branched and linear chains to search for such structures both in cells of the early mouse embryo and in cultured embryonal carcinoma cells throughout differentiation and observed that there is a stage-specific expression of the antigens associated with the branched and the linear structures^{23,24}. The 'branched' antigen was found on mouse embryos from the zygote stage onwards and the 'linear' antigen became detectable at a later stage (5 days after fertilization) in the first differentiated cells, that is, those of the primitive endoderm. This order of appearance of the branched and linear structures contrasts with that in the human erythrocyte membranes, but the branched structures in the early mouse embryo may be formed by the glycosyltransferases of maternal origin in the oocyte; the linear structures, appearing at onset of differentiation, may represent the first fetal products in this series.

Hybridoma-defined carbohydrate antigens

Following observations on the changing expression of the I and i antigens in the early mouse embryo, it was thought (P. W. Andrews and T. F., unpublished) that the hybridoma antibody anti-SSEA-1 against an antigen appearing at the 8-cell-stage of the mouse embryo⁷ might be directed against a carbohydrate structure related to the Ii antigen system. We therefore examined the reactivity of anti-SSEA-1 with a variety of glycoproteins known to express the I and i antigens; we found that anti-SSEA-1 reacts with the intestinal glycoproteins (meconium) of the human fetus²⁵ and used oligosaccharides to inhibit the binding of the anti-embryo antibody, measured by radioimmunoassay. We established^{25,26} that anti-SSEA-1 recognizes α 1 $\rightarrow$ 3 fucosylated *N*-acetylglucosamine (3-fucosyl-*N*-acetylglucosamine; structure 4, Table 1) and deduced that during mouse development, backbone structures of I-type can be converted into SSEA-1 active structures by α 1 $\rightarrow$ 3 fucosylation²⁵. The 3-fucosyl-*N*-acetylglucosamine sequence is not unique to the mouse embryo; it was first discovered in human ovarian cyst glycoproteins²⁷, later found among glycolipids of human adenocarcinomas²⁸ and also in serum glycoproteins and oligosaccharides of human milk

and urine (reviewed in refs 9 and 29). In contrast, this structure in adult mouse occurs only at restricted sites⁷. A second fucose residue joined by $\alpha 1 \rightarrow 2$ linkage to the galactose residue (see structure 6, Table 1) masked completely the expression of the SSEA-1 determinant^{25,29}, suggesting that the sequential addition (or deletion) of monosaccharides might be one mechanism for the appearance, disappearance and reappearance of antigens during stages of embryogenesis and differentiation^{25,30}. The specificity of anti-SSEA-1 was investigated also by structural studies of human erythrocyte glycolipids which contain the SSEA-1 determinant³¹⁻³³ and human gastrointestinal glycolipids³⁴; there is now agreement that this determinant consists of the 3-fucosyl-*N*-acetylactosamine structure. An antibody, D₁ 56-22, raised against a colon cancer cell line, also recognizes this structure³⁴. Furthermore, two or three repeats of this sequence have been detected in some human adenocarcinomas⁹.

A variety of monoclonal antibody-defined differentiation antigens of murine and human haematopoietic or epithelial cells and certain human oncofetal antigens are also carbohydrates, expressed on Type 1 as well as Type 2 backbone sequences or on their additionally glycosylated analogues and may be carried both on glycoproteins and glycolipids (Table 1). Other oncofetal antigens are expressed on carbohydrate chains of the lactosyl-, ganglio- and the globo-series glycolipids (Table 2). Among the structures in Table 1 are those of the major blood group antigens A, B, H, Le^a and Le^b, included first to show their relationship to the antigenic determinants recognized by the anti-mouse

embryo antibody anti-SSEA-1 and the anti-human myeloid cell antibodies VEP8 and VEP9, MY-1 and VIM-D5, the anti-human teratocarcinoma antibody FC 10.2, the anti-human adenocarcinoma/adenoma antibodies 19-9, C14, F3 and D₁ 56-22, and the anti-milk fat globule antibodies M39 and M18. Second, a number of the monoclonal antibodies MH2, NS-10 and the E1 and the CO series which were thought initially to recognize human adenocarcinoma-associated antigens, were later shown to be directed against the blood group antigens ALe^b/Le^y, B, Le^a or Le^b. Presumably, the tumour cells used as immunogens were derived from individuals with the various blood-group genes.

Carbohydrate antigens have different patterns of expression in various animal species. For example, structure 4 (Table 1) recognized by anti-SSEA-1 is a distinctive antigen of granulocytes among human peripheral blood cells^{29,35}, but is not expressed on murine, rat, rabbit or porcine granulocytes³⁶. Moreover, a carbohydrate antigen may be a normal component in one cell type but a tumour-associated antigen in another. Structure 3 (Table 1), recognized by the monoclonal anti-I antibody Ma, is one such example; this antigen is a normal component of human erythrocytes but is a tumour-associated antigen in gastric adenocarcinomas of individuals who secrete the blood group ABH antigens^{37,38}. The biochemical basis of the latter observation may be as follows: the I(Ma) determinant, consisting of the branchpoint sequence Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 6 (see Table 1), occurs abundantly among the oligosaccharides of gastric mucosal glycoproteins and secreted gastric mucins^{37,38}.

Table 1 Differentiation antigens first detected by monoclonal antibodies and identified subsequently as carbohydrate structures based on Type 1 or Type 2 blood group chains

	Structure	Designation of antigen	Cell association
1	Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 3(Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 3) $\geq$ 2	i	Fetal erythrocytes, human ^{17,18} ; primary endoderm, mouse ²³
2	Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcNAc β 1	I	Adult erythrocytes, human ¹⁹ ; preimplantation embryos, mouse ^{23,24}
3	Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 6Gal/GalNAc	I(Ma) M18, M39	Adult erythrocytes, human ⁸¹ ; gastric mucosa, non-secretors; gastric adenocarcinomas, secretors ^{37,38,40} Breast epithelium, human ⁸²
4	Gal β 1 $\rightarrow$ 4GlcNAc $\uparrow$ 1,3 Fuca	SSEA-1 VEP8 and VEP9, Myl, VIM-D5 etc. D ₁ 56-22 (X hapten, Le ^x)	8-Cell stage mouse embryo ^{25,26,33} Myeloid cells, human ^{29,35,83} Colorectal cancer, human ³⁴
5	Gal β 1 $\rightarrow$ 4GlcNAc $\uparrow$ 1,2 Fuca	TRA-1-85 (blood group H)	Human cell lines*
6	Gal β 1 $\rightarrow$ 4GlcNAc $\uparrow$ 1,2 $\uparrow$ 1,3 Fuca Fuca	C14 F3 AH6 (Y hapten, Le ^y)	Colonic adenocarcinoma, human ⁸⁴ Lung adenocarcinoma, human ⁸⁵ Gastric cancer, human ⁸⁶ Embryonal carcinoma cells, human and mouse ⁸⁷
7	GalNAc α 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4/3GlcNAc $\uparrow$ 1,2 Fuca	TL5 (blood group A)	EGF receptor of A431 cells ⁵⁵
8	Gala1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcNAc $\uparrow$ 1,2 Fuca	E ₁ series (blood group B)	Pancreatic cancer ³⁴
9	Gal β 1 $\rightarrow$ 3GlcNAc β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4Glc/GlcNAc	FC10.2	Embryonal carcinoma cells, human ⁸⁸ ; gastric mucosa, non-secretors; gastric adenocarcinoma, secretors ⁴⁰
10	Gal β 1 $\rightarrow$ 3GlcNAc $\uparrow$ 1,4 Fuca	CO-514 (blood group Le ^a)	Adenocarcinomas, human ⁸⁹
11	Gal β 1 $\rightarrow$ 3GlcNAc $\uparrow$ 1,2 $\uparrow$ 1,4 Fuca Fuca	NS-10 CO-43 (blood group Le ^b)	Adenocarcinomas, human ⁸⁹
12	GalNAc α 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 3/4GlcNAc $\uparrow$ 1,2 $\uparrow$ 1,3/4 Fuca Fuca	G49 MH2 (blood group ALe ^b /Le ^y)	EGF receptor of A431 cells ⁹⁰ Colonic adenocarcinoma, human (cited in ref. 53)
13	Gal β 1 $\rightarrow$ 3GlcNAc $\uparrow$ 2,3 $\uparrow$ 1,4 NeuAc Fuca	19.9 (sialyl Le ^a)	Colon cancer, human ³⁹ ; normal pancreatic ducts ³⁴ , normal gastric mucins, non-secretors; gastric cancer mucins, secretors ⁴⁰ , human seminal fluid proteins ⁴¹

* J. K. Picard, P. Goodfellow, P. W. Andrews, G. L. Daniels, K. Uemura & T. Feizi, unpublished observations.

In the majority of the 'secretor' population (whose glycoprotein secretions express the blood group A, B or H antigens of their erythrocytes) the antigenicity of this structure is normally masked by the blood group A, B or H monosaccharides. On the other hand, this antigen is expressed strongly in the mucosal glycoproteins of 25% of the 'non-secretor' population³⁷; in these individuals, the I(Ma) determinant presumably is not further glycosylated by the blood group H, A or B monosaccharides and remains accessible. When adenocarcinomas arise in the gastric mucosae of 'secretors', the I(Ma) determinant behaves as a tumour-associated antigen, presumably because the blood group chains are synthesized incompletely.

The sialylated blood group Le^a, (structure 13, Table 1) defined by the hybridoma antibody 19.9 is another tumour-associated antigen found in certain tissues of certain individuals, but a normal antigen in others. This antigen is expressed strongly on glycolipids and glycoproteins extracted from adenocarcinomas of the colon, but not on those of the normal colon^{34,39,40}, but it has been detected among normal pancreatic glycolipids³⁴ and normal seminal fluid glycoproteins⁴¹. In glycoproteins extracted from gastric mucosae, this antigen (like the I(Ma) and FC10.2 antigens), however, is a normal component in 'non-secretors' and a tumour-associated antigen in 'secretors'⁴⁰. The enzymatic basis of these antigenic changes requires investigation, particularly at the level of glycosyltransferase genes.

Apart from carbohydrate determinants shared commonly by glycoproteins and glycolipids, some structures which occur predominantly on glycolipids of the lactosyl, ganglio and the globo series (Table 2), have been shown also to behave as onco-developmental antigens recognized by hybridoma antibodies. These include: (1) the *N*-acetylneuraminic acid form of G_{D3} (structure 15, Table 2), recognized by the hybridoma antibodies R₂₄ and 4.2 raised against human melanoma cell lines; (2) the 9-*O*-acetyl-*N*-acetylneuraminic acid form of G_{D3}, (structure 16, Table 2), recognized by antibody D1.1 raised against a neuroectodermal antigen of fetal rat and expressed also on human melanoma cells; (3) the blood group P^k determinant, (structure 19, Table 2), recognized by hybridoma 38.13 raised against Burkitt lymphoma cell lines; and (4) two epitopes on structure

21 (Table 2) behaving as the stage-specific embryonic antigens SSEA-3 and SSEA-4 of mouse embryos.

Thus, numerous studies have assigned to carbohydrate structures roles as differentiation antigens and oncofetal antigens. These structures are not confined to any one normal or neoplastic cell type. All such antigens detected by monoclonal antibodies and characterized at the determinant level have been associated with saccharides. These new observations with monoclonal antibodies agree with current knowledge about the genetics and structures of the blood group antigens and their precursors^{15,42,43} and the differences in the carbohydrate moieties of the glycolipids of normal and neoplastic cells^{9,44} and of various types of differentiated cells⁴⁵. These differences presumably are the end results of either differences in the activities of glycosyltransferases, or in the availability of their substrates, or differences in glycosidase activities in various cell types. Thus, the hope that unique antigenic markers of tumour cells would be discovered and revolutionize tumour diagnosis and therapy is being replaced by the realization that such antigens may not exist.

The failure to discover unique antigenic markers of developmental stage or neoplastic state may reflect limitations in the methods of selection of hybridoma antibodies. This is not necessarily so, for certain autoantibodies and monoclonal anti-tumour antibodies produced by lymphocyte cell lines derived from patients with neuroectodermal tumours or melanomas are directed also at carbohydrates (structures 17 and 18, Table 2). Thus, the possibility must be considered seriously that: (1) saccharides are the much-sought surface markers that distinguish immature from mature cells and tumour cells from their normal counterparts; (2) the carbohydrate sequences, and/or some additional structures associated with them, may have specific roles in normal cell growth and differentiation; (3) the inappropriate biosynthesis or processing of carbohydrate structures may contribute to the disordered behaviour of tumour cells.

Role of saccharide antigens

Most of these carbohydrate antigens have unknown function. Interestingly, a high proportion of the hybridoma-defined anti-

Table 2 Differentiation antigens first detected by monoclonal antibodies or autoantibodies and identified subsequently as carbohydrate structures of glycolipids of the lactosyl, ganglio or globo series

	Structure	Designation	Cell association
Lactosyl series			
14	Galβ1 → 4Glc-Cer	T ₅ A ₇ (lactosylceramide)	Myeloid cells, human ⁹¹
15	Galβ1 → 4Glc-Cer ↑ _{2,3} NeuAcα ↑ _{2,8} NeuAcα	R ₂₄ 4.2 (G _{D3})	Melanoma, human ^{92,93}
16	Galβ1 → 4Glc-Cer ↑ _{2,3} NeuAcα ↑ _{2,8} Neu5,9Ac ₂ α	D1.1	Neuroectoderm, rat; melanoma, human ⁹⁴
Ganglio series			
17	GalNAcβ1 → 4Galβ1 → 4Glc-Cer ↑ _{2,3} NeuAcα	OFA-1 (autoantigen) (G _{M2})	Melanoma and fetal brain, human ⁹⁵
18	GalNAcβ1 → 4Galβ1 → 4Glc-Cer ↑ _{2,3} NeuAcα ↑ _{2,8} NeuAcα	OFA-2 (autoantigen) (G _{D2})	Melanoma, human ⁹⁶ Neuroectoderm, human ⁹⁷
Globo series			
19	Galα1 → 4Galβ1 → 4Glc-Cer	38.13 (blood group P ^k)	Burkitt lymphoma cell lines ⁹⁸
20	GalNAcα1 → 3GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glc-Cer	M1:22:25:8 (Forssman)	Embryos and embryonal carcinoma cells, mouse ^{3,4}
21	NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glc-Cer SSEA-4 SSEA-3	SSEA-3, SSEA-4	4- To 8-cell-stage embryos, mouse ^{99,100}

gens thus far elucidated are related to the blood group antigen system, possibly reflecting the ease of 'recharacterization' of known structures. Even the amounts of the major blood group antigens A, B and H, in organs of the human fetus change in successive stages of development⁴⁶; this suggests that these carbohydrate structures also are differentiation antigens, but their physiological roles are still unknown. Among some other unexpected developments is the demonstration that most monoclonal antibodies against the receptor for epidermal growth factor (EGF) on the epidermoid carcinoma cell line A431 show specificity for blood group-related carbohydrate structures (reviewed in refs 47, 48, 102). Blood-group and related antigens are expressed strongly on the surface of the epidermoid carcinoma cell line A431 (refs 47, 49); the EGF receptor seems to be the major blood group active glycoprotein in this cell line. One of the monoclonal antibodies (TL5) raised against the EGF receptor of A431 cells⁵⁰ recognizes the blood group A antigen⁴⁹ and may behave as a growth factor agonist in certain conditions. This antibody does not compete with EGF nor does it have a direct stimulatory effect on fibroblasts, but in an experiment where the cell-bound antibody was crosslinked in the presence of anti-mouse antibodies, the fibroblasts took up tritiated thymidine⁵⁰. If this stimulatory effect is mediated by perturbation of blood group A-active carbohydrate chains, blood group structures are likely to be receptors for certain (as yet undefined) regulators of cell proliferation⁴⁸, and systematic studies on the roles of this family of saccharides in the control of cell growth would be encouraged.

One approach to elucidate the roles of the developmentally-regulated carbohydrate structures (such as blood group-related carbohydrate chains based on Type 1 and Type 2 backbones) is to identify the cellular glycoproteins that carry them. This provided the first evidence⁵¹ for their occurrence on the anion transport protein (band 3) of erythrocyte membranes and on high- M_r glycoproteins, including the leukocyte common antigen of human B lymphocytes and the sialoglycoproteins of human T lymphocytes^{52,53}, just as for the EGF receptor glycoprotein⁴⁷. Well-characterized monoclonal antibodies may be used increasingly for studies of the carbohydrate moieties of glycoproteins and glycolipids.

Another approach is to perturb cell interactions during early embryogenesis (for example, those leading to the process of compaction) by using structurally-defined oligosaccharides as inhibitors. A reversal of compaction of mouse embryos with oligosaccharides expressing the SSEA-1 determinant has been reported⁵⁴. In another study this was not found with free oligosaccharides⁵⁵, but an inhibition of compaction in the presence of a trivalent conjugate of this carbohydrate sequence was found⁵⁵. An alternative approach, treating embryos with endo- β -galactosidase, provides evidence for the involvement of carbohydrate chains of poly- N -acetylactosamine type in the compaction process⁵⁶. Compacting embryos (at the 8- to 16-cell stage) decompacted experimentally in calcium-free medium and treated with endo- β -galactosidase, have a dramatically prolonged recompaction time. Endo- β -galactosidase cleaves specifically long-chain oligosaccharide backbones containing the sequence $\text{GlcNAc}(\beta 1 \rightarrow 3\text{Gal}\beta 1 \rightarrow 3/4\text{GlcNAc})_n$, abundant on embryonic cells²²; it abolishes the SSEA-1 activity associated with them⁵⁶.

A role for a specific carbohydrate structure in the metastatic properties of B16 melanoma cells of mouse could be that mutant cell lines with reduced metastasizing ability and an increased cell-to-cell adhesive property contain elevated concentrations of $\alpha 1 \rightarrow 3$ -fucosyltransferase and an increased surface expression of the SSEA-1 determinant⁵⁷. The purified glycosyltransferase can act as a mediator of the adhesion of human fibroblasts⁵⁸. These observations suggest that adhesion and reduced metastasis are mediated by the interaction of this glycosyltransferase on the surface of B16 mutant cells with acceptor substrates on other cells. These and some other observations on galactosyltransferase activities on the surface of embryonal carcinoma cells⁵⁹ support the hypothesis⁶⁰ that glycosyltransferases

of cell surfaces may mediate cell-to-cell adhesion by interacting with acceptor substrates on neighbouring cells. *In vitro* experiments with immobilized α -mannosidase indicate that glycosidases also may serve as cell adhesion molecules⁶¹.

Biological functions of carbohydrates

Biochemical (rather than immunochemical) experiments have allowed the assignment of roles to specific saccharide structures, all of which are potential antigens. For example, the blood anticoagulant activity of heparin results from its ability to bind with high affinity to the protease inhibitor anti-thrombin. This binding is mediated by a specific pentasaccharide sequence on the heparin molecule⁶². There is evidence also for important biological activities associated with other, non-anticoagulant oligosaccharide sequences in heparin: (1) the promotion of angiogenesis by intact heparin and the inhibition of angiogenesis by a hexasaccharide fragment derived from it⁶³ and (2) the inhibition of smooth muscle proliferation by an oligosaccharide fragment derived from the heparin of endothelial cells⁶⁴. I suggest that the latter observation raises the important possibility that this heparin oligosaccharide sequence may be a receptor analogue for platelet-derived growth factor (PDGF). Some important functions have also been ascribed to structurally-defined sialoglycolipids (gangliosides). The ganglioside GQ_{1b} enhances greatly neurite outgrowth and cell proliferation in neuroblastoma cell lines⁶⁵. Gangliosides GM_1 and GM_3 may modulate the phosphorylation (and hence the function) of the receptor for PDGF in a mouse fibroblast (3T3) cell line⁶⁶. These are strong indicators of the importance of carbohydrate structures in the regulation of cell growth.

The role of terminal galactose residues of asialoglycoproteins as recognition structures for their clearance from serum by uptake into hepatocytes⁶⁷ and the importance of phosphorylation of mannose residues of lysosomal enzymes for their correct routing to lysosomes⁶⁸ are well established. This latter observation indicates clearly that modification of oligosaccharide sequences, for example, by phosphorylation, may change greatly their biological properties.

An important function assigned to specific oligosaccharide sequences in plants has been the eliciting of antimicrobial compounds, phytoalexins. There is strong evidence to suggest that the active substances are oligosaccharide fragments released from the injured plant-cell walls and from the cell walls of fungi infecting the plant roots (reviewed in ref. 69). The corresponding carbohydrate binding molecules have not been identified. On the other hand, a variety of carbohydrate binding proteins (lectins) are distributed widely in plant and animal cells^{70,103}. Although the precise functions of these lectins, in their tissues of origin are unknown, the biological effects of plant lectins on animal cells, for example, the induction of blast transformation of lymphocytes by phytohaemagglutinin, have been studied widely⁷⁰. In transformed and mitogen-stimulated lymphocytes, we have detected¹⁰⁴ increased levels of proteins antigenically related to an animal lectin (soluble β -galactoside-binding lectin). Thus, it is possible that the lectin and related proteins are somehow involved in the regulation of cell growth. In fibroblasts and epithelial cells, we (S. R. Carding and T.F., cited in ref. 104) have detected several potential receptors for this lectin in the M_r range 10,000–180,000. It will be important to investigate whether growth factor receptors, such as the EGF receptor which contains carbohydrate chains with β -galactoside termini⁴⁷, interact with this and other endogenous lectins within or on the surface of cells.

As well as having roles as recognition structures for endogenous ligands, carbohydrate sequences of cell surfaces serve as receptors for infective agents and their toxins^{71–75}. They may be important determinants of the tropisms of diverse infective agents. Furthermore, various strains of influenza virus have binding specificities for different sialic acid $\rightarrow$ galactose linkages and extracellular (soluble) sialoglycoproteins of the host may exert selective pressures resulting in the emergence of variant viruses with altered cell-binding specificities⁷⁶. Thus, the

well-known properties of serum glycoproteins in inhibiting the attachment of viruses to cells and the differing sialyloligosaccharide sequences on the glycoproteins of different animal species may be important in the ecology of the influenza virus.

Studies on the adhesive specificity of the human pathogen *Mycoplasma pneumoniae* indicate that the autoimmune manifestation following infection (the production of high-titre autoantibodies to the erythrocyte antigen I) has its origin in the interaction of the infective agent with saccharide receptors of the host cells, conclusions reached following the observation that the receptors for this agent on human erythrocyte membranes are sialyloligosaccharides of Ii antigen type⁷⁷. Thus, the post-infective anti-I autoantibodies can be considered 'anti-receptor' antibodies directed against the backbone structures of the receptor sequence, raising the possibility that the formation of complexes between the adhesive molecules of infective agents and specific saccharides of host-cell membranes may be an important mechanism for eliciting autoantibodies. The same principle may apply to other autoimmune disorders with anti-receptor antibodies where the causative agents are not yet known but infective agents are not excluded. In those instances where host-cell receptors are saccharide structures shared by several glycoproteins and glycolipids, as with *M. pneumoniae*, there will be cell-type and species differences in the receptor macromolecules⁷⁸, reflecting the differences in glycosylations. Hence, the biological effects of a given infective agent will differ accord-

ingly. Presumably, such cell-type differences account for the different effects of *M. pneumoniae* on human T and B lymphocytes; *M. pneumoniae* triggers a mitogenic response in T lymphocytes but induces immunoglobulin secretion without mitogenesis in human B lymphocytes⁷⁹. I suggest that many mechanisms of tropism and some of the transformation-related phenomena of oncogenic viruses are a function of their carbohydrate-binding (lectin-like) proteins.

Conclusions

Monoclonal antibodies have not revealed the much-sought, unique antigen markers of embryonic stage and neoplastic state; few of them may survive the critical assessment of the oncologist for the purpose of tumour diagnosis and therapy⁸⁰. The antibodies characterized already and those awaiting characterization, however, are reagents which will become fundamental analytical tools for future studies of the glycoconjugates of whole cells and their receptors. Although much progress is being made on the structural analysis of glycoconjugates by physicochemical procedures, these are unlikely to supersede the sensitivity of monoclonal antibodies for visualizing the *in situ* disposition of the diverse oligosaccharide sequences in individual cells.

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Importance of the 'shape' of the melting regime during partial melting of the mantle

M. J. O'Hara

Geology Department, University College of Wales, Aberystwyth, Dyfed SY23 3DB, UK and
Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California 91125, USA

The amount of melt formed in individual stream lines of upper mantle rocks passing through a partial melting regime increases from zero at the edge to a maximum at the central axis. Relationships between aggregated liquid compositions from such melting regimes and the source mantle should be obtained by integration of the appropriate equations for liquid composition taking into account the horizontal and vertical form of the melting regime. Misinterpretation of integrated liquid products from such regimes would be easy if too simple a model of the melting process is used.

GEOCHEMICAL effects of partial melting of upper mantle rocks have been explored over the past 16 years using relationships developed by Gast¹, Shaw² and Langmuir *et al.*³. Applications of these relationships to the prediction or interpretation of data sets for volcanic rocks have assumed implicitly that all parcels of the mantle processed through the partial melting regime undergo an equal amount of melt removal. Yet it was known throughout this time that the maximum temperature above the solidus and, therefore, the mass fraction of melt developed in parcels of mantle passing through the melting regime, will be at a maximum along the central axis and will decline to zero at the edges⁴.

Consider one example of the consequence of using more sophisticated models—the case where mass fraction (F) of melt developed on each stream line increases to some central maximum (F_m) as a linear function of distance inward from the periphery, in a melting regime of semi-infinite lateral extent. Only half as much liquid will be developed in this first case as would be developed in a second, or 'simple', case where all the mantle which had encountered the melting regime underwent the same (maximum F_m) mass fraction of partial melting.

Now consider a totally incompatible element, wholly concentrated in the first drop of liquid to form along each stream line. The concentration of this element in the liquid derived in the first case will necessarily be just twice its concentration in the liquid derived in the second case. If the melting regime were circular in plan (for example, a plume) rather than of semi-infinite linear extent (for example, a mid-ocean ridge segment) the amount of liquid developed will be one-third that developed in the 'simple' case and the concentration of the totally incompatible element consequently will be three times that in the liquid derived in the 'simple' case.

Most attempts to invert the geochemistry of erupted basalt magmas to predict upper mantle chemistry, or to predict potential basalt compositions which might form by partial melting of particular mantle materials, have used the equations¹⁻³ which relate liquid, residue and source mantle compositions in the 'simple' case mentioned above. Clearly, when this is done inappropriately, there will be a tendency towards some combination of: (1) underestimation of the mass fraction of melting involved relative to the maximum amount actually developed (F_m); and (2) overestimation of the content of the totally incompatible element in (that is, the 'fertility' of) the source mantle.

Adoption of the more sophisticated melting model allows an immediate reconciliation between field, petrographic and major element chemical features in upper mantle materials which point to large ($F_m \sim 0.25-0.35$) maximum mass fractions of melt removal (see, for example, ref. 5) and features of trace-element geochemistry which seem to point to very small mass fractions

of partial melt removal (see, for example, ref. 6). The incompatible trace-element chemistry of the aggregated melts is dominated by liquid contributions from the periphery of the melting regime, where the mass fraction of melting is indeed small, whereas events in the central region tend to dominate the other aspects mentioned.

Adoption of the more sophisticated model has some relevance to the interpretation of the apparently higher 'fertility' (deduced using 'simple' models) of the mantle sources⁷ which feed 'plume' type volcanism. Eventually, this model may reconcile the apparent fertility of plumes with the undeniable fact that significantly fertile mantle is too iron-rich, too well endowed with spinel and garnet and hence too dense to rise through residual mantle⁸⁻¹¹. In principle, the sophisticated model permits a less fertile mantle material, rising in a circular plan-form plume, to yield a liquid more enriched in incompatible elements than is obtained from neighbouring more fertile mantle undergoing melting in a linearly extensive melting regime.

Geochemical features suggestive of mixing of magmas from two or several sources when 'simple' models are used^{12,13} may derive at least in part from the fact that in the sophisticated model the primary magmas are indeed complex mixed melts.

It may seem that the trace-element geochemistry of erupted basalts can still be inverted viably to yield upper mantle source characteristics using the 'simple' relationships with some average value for the mass fraction of liquid developed from the whole mass of mantle passing through the melting regime. But this is only true for totally incompatible elements.

Integration of the relationships described below yields expressions which in turn predict the liquid and residue compositions that might be produced in sophisticated melting regimes. These compositions cannot be inverted safely for elements which are not totally incompatible, using any average value of the mass fraction of partial melting inserted into the 'simple' relationships. Highly incompatible element concentrations predicted by the sophisticated model are lower than expectations from the best fit 'simple' model using a single average value of the melt fraction.

Discrimination of concentration between two highly incompatible elements of very similar distribution coefficient is achieved much more efficiently in the melts predicted by the sophisticated model. In 'simple' models, such discrimination can only be produced at very small mass fractions of partial melting. Interpretations based on 'simple' models (which may not be appropriate) may lead towards erroneous conclusions that particular magmas have been derived by exceptionally small mass fractions of partial melting or by extraction from mantle sources which have been depleted of their most highly incompatible elements in a previous small-scale melt extraction event.

Aggregated melt composition relationships

The treatment which follows is simplified greatly; it is used to explore some first-order effects which will result from the adoption of more sophisticated models of melting regimes, and is considered as an intermediate advance rather than a conclusion. (Several points for further refinement are noted in passing.) Partial melting is assumed here to result from the decompression of hot mantle ascending along an adiabat intersecting the solidus curve⁴, but this assumption is not critical in the mathematical formulation.

The 'shape' of the melting regime has two components. The plan-form 'shape' is assumed to be either linear or circular (but solutions for the elliptical end-form would be desirable). It is further assumed that the amount of melt extracted is a maximum on the central axis or at the centre and falls off symmetrically towards the periphery. All melt extracted is assumed to be aggregated and tapped at the central axis only (tapping of liquids developed from sub-portions of a large melting regime has obvious relevance to the Galapagos Islands and sea mounts near spreading centres).

The cross-section 'shape' of the melting regime is defined here by the relationship between distance, y , from the edge of the melting regime (Fig. 1) relative to Y_m , the distance to the centre of the melting regime and F_y , the maximum mass fraction of melt extracted from a parcel of mantle at distance y from the edge, relative to F_m (the maximum mass fraction of melt extracted from a parcel of mantle passing along the central axis of the regime). Melting of sub-spherical blobs is not addressed here.

The 'parcel' of mantle is defined as a very small mass or volume of mantle of width dy (unit length perpendicular to the cross section) and height dh (such that an additional mass fraction of partial melt, dF_y , is produced within it as it moves upwards a distance, dh , along the stream line).

The appropriate relationships have then been integrated¹⁴ on the following assumptions: First,

$$(y/Y_m) = (F_y/F_m)^n \quad (1)$$

where $n > 0$ (alternative assumptions for the relationship might yield a more easily integrated expression, advantageous because it would allow inversion of data sets to obtain the important parameters F_m and n).

Second, the partial melting process affecting each individual parcel of mantle produces a continuous partial melt (CPM), a process in which some mass fraction of the residue is trapped liquid, but in which further liquid is drained continuously from the parcel as melting proceeds. Each new infinitesimal increment of liquid formed is well mixed with the trapped liquid and balanced by the infinitesimal decrement of melt removed from that mantle parcel at the same step. The process has been modelled using the familiar relationship² for the composition of a perfect fractional partial melt (PFPM).

$$C_L/C_S = \frac{(1-f_y)^{1/D^*-1}}{D^*} \quad (2)$$

C_L is the concentration of an element in the liquid present in (or removed from) the parcel at the stage when mass fraction f_y of the original parcel has been removed by the CPM process (this parcel of mantle will yield ultimately a maximum mass fraction of melt F_y , where $F_y < F_m$). C_S is the concentration of the element in the original mantle parcel before the onset of melting and melt removal. The effects of trapped liquid are taken into account in the formulation of the bulk distribution coefficient, D^* as

$$D^* = D(1-z) + z \quad (3)$$

where D = concentration of element in the bulk crystal assemblage divided by the concentration of element in the liquid and z is the mass fraction of the trapped liquid residue. (Trapped liquid is treated simply as another residual phase for which the distribution coefficient happens to be unity.)

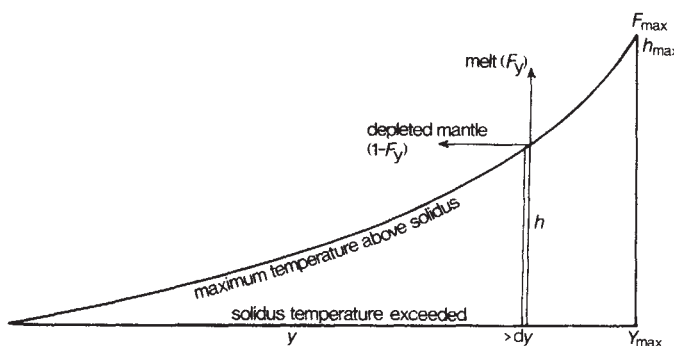


Fig. 1 Half cross-section of a hypothetical upper mantle melting regime (adapted and simplified from ref. 4) illustrating the relationships between the distance y of a stream-line, height h , in the partial melting regime and Y_m , the distance to the centre. The mass fraction F_y of melt extracted along this stream line is assumed proportional to h and the 'shape' of the melting regime is defined by the relationship between y and F_y (see text). Arrows labelled 'depleted mantle' and 'melt' are stylized and indicate no more than physical separation of melt from residue somewhere in the regime.

Equations (2) and (3) yield a precise description of the process at all values of D , z and f_y (full integration from mass balance equation on request from the author) and no problems arise with melt not collected from the periphery because f_y , F_y and F_m are defined as the mass fractions of melt which are collected.

Third, z , D and hence D^* are assumed constant for all parcels of mantle throughout the melting process and C_S is also assumed constant in time and space. (Subsequent developments must examine the consequences of the variations in D which accompany both the changes in the proportions of the crystal species which will occur when F_m is large and the changes in phase assemblage and liquid structure which will occur when the melting regime extends through a significant range of pressure. In principle, these complications can be introduced into the numerical integration of the relationships derived below, but the assumptions made here are necessary for the analytical solutions presented.) Note, however, that for very highly incompatible elements with $D < 0.005$, the value of D^* will be dominated by the mass fraction of trapped liquid, z , if this is greater than ~ 0.005 (0.5%).

Fourth, all decrements of melt removed from all parcels of mantle are aggregated without any further interaction with source material or residues, and well mixed to form an accumulated perfect fractional partial melt (APFPM) or an accumulated continuous partial melt (ACPM). The composition of the APFPM or ACPM derived from a single mantle parcel during its passage through the melting regime, or from all parcels along a single mantle stream-line through the regime at one instant, is then obtained from the familiar relationship²

$$\bar{C}_L/C_S = \{1 - (1 - F_y)^{1/D^*}\}/F_y \quad (4)$$

which is the integral of equation (2) between $f_y = 0$ and $f_y = F_y$.

Fifth, residue stream-line patterns and F_y along each flow line are assumed to remain constant with time (that is, the process is in a steady state).

Sixth, the APFPMs or ACPMs from each mantle stream line are in turn aggregated to yield a complex accumulated perfect fractional (or continuous) partial melt (CAPFPM or CACPM) which constitutes the primary magma segregated from the partial melting regime. The relationship between the concentration of an element $\bar{C}_L$ in this CAPFPM or CACPM and its concentration in the source C_S , is obtained by integrating $\bar{C}_L/C_S$ (equation 4) for $F_y = 0 \rightarrow F_m$ over the cross-section shape and along (linear) or around (circular) the plan-form of the melting regime, as

follows:

$$\begin{aligned} \bar{C}_L/C_S = & \left\{ \int_0^{F_m} \left[\begin{array}{l} \text{relative length in planform} \\ \text{to which result applies} \end{array} \right] \right. \\ & \times \left[\begin{array}{l} \text{concentration of element} \\ \text{in liquid increment} \end{array} \right] \\ & \times \left[\begin{array}{l} \text{mass fraction of liquid} \\ \text{having this concentration} \end{array} \right] dy \Big\} \\ & \div \left\{ \int_0^{F_m} \left[\begin{array}{l} \text{relative length in planform} \\ \text{to which result applies} \end{array} \right] \right. \\ & \times \left[\begin{array}{l} \text{mass fraction of liquid} \\ \text{having this concentration} \end{array} \right] dy \Big\} \end{aligned} \quad (5)$$

In the linear planform case the relative lengths in planform are all the same, hence the first bracket top and bottom becomes unity. In the circular planform case the relative lengths are given by $2\pi(1 - y/Y_m)$ which is $2\pi(1 - (F_y/F_m)^n)$. The middle bracket on the top line contains the expression from equation (4) above. The last bracket, top and bottom, is simply F_y .

Substituting, from equation (1), $dy = (nY_m/F_m)F_y^{n-1}dF_y$ and cancelling constants n , F_m , Y_m in top and bottom lines, one obtains in the linear planform case

$$\frac{\text{CAFFPM}}{\text{LINEAR}} \frac{\bar{C}_L}{C_S} = \frac{\int_0^{F_m} \left[\frac{1 - (1 - F_y)^{1/D^*}}{F_y} \right] F_y F_y^{n-1} dF_y}{\int_0^{F_m} F_y F_y^{n-1} dF_y} \quad (6)$$

or, in the circular plan case

$$\frac{\text{CAFFPM}}{\text{CIRCULAR}} \frac{\bar{C}_L}{C_S} = \frac{\int_0^{F_m} \left[1 - \left(\frac{F_y}{F_m} \right)^n \right] \left[\frac{1 - (1 - F_y)^{1/D^*}}{F_y} \right] F_y F_y^{n-1} dF_y}{\int_0^{F_m} \left[1 - \left(\frac{F_y}{F_m} \right)^n \right] F_y F_y^{n-1} dF_y} \quad (7)$$

Both expressions may be further simplified by cancellation of an F_y in the top line and summing powers of F_y in the bottom line before commencing numerical or analytical integration.

Seventh, the derivation of equations (5) and (6) assumes that each decrement of melt removed from each parcel of mantle undergoes no further chemical interaction with any other parcel of mantle or residue, but this represents only one extreme possible assumption. Another (not the only other) extreme assumption is that each decrement of melt flows upward along the mantle stream line, remaining in perfect equilibrium with the residues, and is then aggregated. This leads to the alternative relationship to that given in equation (4),

$$\bar{C}_L/C_S = 1/[D^* + F_y(1 - D^*)] \quad (4')$$

which is familiar² as a form of the relationship for the composition of a perfect equilibrium partial melt (PEPM) product developed where mass fraction F_y of the original source has become removable melt. That this is true can be demonstrated by integration of the appropriate mass balance equations, but may also be appreciated intuitively. All the melt (mass fraction F_y) from the whole stream line must pass through and equilibrate with the final residue, mass fraction $(1 - F_y)$ leading to equation (4') by simple mass balance. (Note the separate increments of melt composing the mass fraction F_y commenced their travel at different times). Equation (4') is valid provided n and F_m are constant, which is not guaranteed in real geological situations.

Integration as before then yields the alternative expressions for the linear plan case

$$\frac{\text{CAFFPM}}{\text{LINEAR}} \frac{\bar{C}_L}{C_S} = \frac{\int_0^{F_m} \left[\frac{1}{D^* + F_y(1 - D^*)} \right] F_y F_y^{n-1} dF_y}{\int_0^{F_m} F_y F_y^{n-1} dF_y} \quad (6')$$

and for the circular plan case,

$$\frac{\text{CAFFPM}}{\text{CIRCULAR}} \frac{\bar{C}_L}{C_S} = \frac{\int_0^{F_m} \left[1 - \left(\frac{F_y}{F_m} \right)^n \right] \left[\frac{1}{D^* + F_y(1 - D^*)} \right] F_y F_y^{n-1} dF_y}{\int_0^{F_m} \left[1 - \left(\frac{F_y}{F_m} \right)^n \right] F_y F_y^{n-1} dF_y} \quad (7')$$

Computation of the expressions in equations (6), (7), (6') and (7') is best carried out by numerical integration, particularly for the case of very highly incompatible elements with low values of D^* (<0.003). Analogous expressions may be written to describe the average residue compositions¹⁴.

However, integration of equations (6) and (7) by substitution by the chain rule and by parts, yields in the general case, an infinite series¹⁴ which may be rearranged in the forms given in equations (8) and (9) below for the linear and circular plan forms respectively.

$$\begin{aligned} \frac{\text{CAFFPM}}{\text{LINEAR}} \frac{\bar{C}_L}{C_S} = & \left[\frac{n+1}{n} \right] \left\{ \left[\frac{1 - (1 - F_m)^{1/D^*}}{F_m} \right] \right. \\ & \left. - \left[\frac{(1 - F_m)^{1/D^* - 1}}{D^*} \right] \{\text{series 1}\} \right\} \end{aligned} \quad (8)$$

where

$$\begin{aligned} \{\text{series 1}\} = & \left\{ \frac{1}{(n+1)} + \frac{F_m}{(1 - F_m)D^*} \frac{(1 - D^*)}{(n+1)(n+2)} + \dots \right. \\ & \left. + \frac{Q^{p-1}n!(1 - D^*)(1 - 2D^*) \dots (1 - (p-1)D^*)}{(n+p)!} \right\} \end{aligned}$$

and where $Q = [F_m/(1 - F_m)D^*]$ and the general term is written for the p th term of the subseries, designated here as {series 1}.

$$\begin{aligned} \frac{\text{CAFFPM}}{\text{CIRCULAR}} \frac{\bar{C}_L}{C_S} = & \left[\frac{(n+1)(2n+1)}{2n^2} \right] \\ & \times \left\{ \left[\frac{1 - (1 - F_m)^{1/D^*}}{F_m} \right] - \left[\frac{(1 - F_m)^{1/D^* - 1}}{D^*} \right] \right. \\ & \left. \times [2\{\text{series 1}\} - \{\text{series 2}\}] \right\} \end{aligned} \quad (9)$$

where

$$\begin{aligned} \{\text{series 2}\} = & \left\{ \frac{1}{2n+1} + \frac{Q(1 - D^*)}{(2n+1)(2n+2)} + \dots \right. \\ & \left. + \frac{Q^{p-1}(2n)!(1 - D^*)(1 - 2D^*) \dots (1 - (p-1)D^*)}{(2n+p)!} \right\} \end{aligned}$$

The term $(1 - (p-1)D^*)$ in the general term of these series must eventually become negative for $D^* > 0$ and successive terms thereafter are alternately positive and negative. The series will converge provided $F_m/(1 - F_m) < 1$, that is, $F_m < 0.5$ (the majority of geologically reasonable cases) and evaluation will terminate, with all later terms being zero, at the $(1/D^* + 1)$ th term if $1/D^*$ is an integer.

Equations (8) and (9) may be simplified for analytical purposes to

$$\left[\begin{array}{c} \text{Complex} \\ \text{aggregated} \\ \text{melt} \end{array} \right] = \left[\begin{array}{c} \text{shape} \\ \text{factor} \end{array} \right] \left\{ \left[\begin{array}{c} \text{APFPM} \\ \text{at } F_{\max} \end{array} \right] - \left[\begin{array}{c} \text{PFPM} \\ \text{at } F_{\max} \end{array} \right] \left[\begin{array}{c} \text{series} \\ \text{factor} \end{array} \right] \right\} \quad (10)$$

Equation (10) may be stated as follows: The concentration of an element in a CAFFPM or CACPM product will be that expected in an APFPM (or ACPM) product developed at the maximum mass fraction of partial melting minus the concentration of that element developed in the last extracted drop of a PFPM (or CPM) product at the same maximum mass fraction of melting multiplied by a series term whose value is a function of F_m , D^* and the power function n ; the whole of the above multiplied by a shape factor which is a function of the power n alone.

Evaluation of the relationships

Some important properties of equations (8) and (9) are: First, when $D^* = 0$ (totally incompatible element), $\bar{C}_L/C_S = [\text{shape}$

factor][$1/F_m$]. Second, when $D^* = 1$, $\bar{C}_L/C_S = 1$ (logical). Third, when $D^* \rightarrow \infty$ (totally compatible element), $\bar{C}_L/C_S \rightarrow 0$ (logical). Fourth, when $0 < D^* < 1$, $\bar{C}_L/C_S$ is less than would be expected in a simple APFPM product at the appropriate F_m multiplied by the shape factor. The quantity by which it is less is given by [PFPM at F_m] {series factor} [shape factor]. As D^* becomes smaller, the [PFPM at F_m] term passes through a maximum where $D^* = F$ and thereafter becomes very rapidly smaller. Simultaneously, the {series factor} increases in magnitude. The net effect is that this subtracted quantity passes through a maximum (and $\bar{C}_L/C_S$ through a minimum relative to the simple APFPM term) at some intermediate value of D^* which is a function of F_m and n . The resultant values of $\bar{C}_L/C_S$ nevertheless seem to discriminate increasingly efficiently between elements with very small but fixed differences of D^* as D^* itself decreases.

Fifth, as $n \rightarrow 0$ (the mass fraction of partial melting is small over most of the regime but increases sharply close to the central axis), the [shape factor] $\rightarrow \infty$ and $\bar{C}_L/C_S$ tends to very large values when $D^* < 1$, very small values when $D^* > 1$. The parameters $n = 1$, $D^* = 0$ describe the case discussed in the introduction. Sixth, when $n \rightarrow \infty$ (the mass fraction of melt extracted is very close to F_m for all flow lines through the regime) the differences between equations (8) and (9) disappear since all terms in {series 1} and {series 2} tend to zero. The [shape factor] $\rightarrow 1$, and $\bar{C}_L/C_S \rightarrow [1 - (1 - F_m)^{1/D^*}]/F_m$ which in the limit is identical with equation (4).

Seventh, the [APFPM] term in equation (10) yields concentrations which differ little from those of PEPM (see equation 4'), particularly when F_m is substantial. When F_m is substantial (0.2–0.4), this term is very inefficient at discriminating between very highly incompatible elements with small differences of D^* . To obtain good discrimination using this term, it is necessary to reduce F_m to values ~ 0.01 rather than ~ 0.3 .

Eighth, the [PFPM] term in equation (10) yields results which differ dramatically from the [APFPM] term when D^* is small and F_m is substantial. Moreover, the [PFPM] term discriminates very efficiently between very highly incompatible elements of very similar D^* , but at the cost of generating minute concentrations of these elements in the [PFPM] product when F_m is substantial. For very highly incompatible elements this term would be insignificant were it not for the multiplier contained in the series term (see below).

Finally, the {series factor} in equation (10) may contain some very large terms, particularly when F_m is large and D^* and n are small. When D^* is small, the largest terms in the series are grouped about $1/2D^*$ terms into the series; all the first $1/D^*$ terms are positive and all other terms are small (for example, if $D^* = 0.001$, the largest terms contributing to the {series factor} are ~ 500 terms into the series, and involve evaluation of quantities such as $Q^{499} \approx 400^{499}$; $(n+p)! \approx 501!$).

Geological significance

These and other properties¹⁴ of equations (8), (9) and (10) have important implications for the interpretation of major and trace element geochemistry and experimental petrology data for natural lavas, which include:

(1) Even true primary magmas (developed according to the above scenario) would not be in phase or chemical equilibrium with any specific residue composition (although the discrepancy may be small in some respects); nor would the bulk CAPFPM product be in (conventional) chemical equilibrium with the bulk residue from the process.

(2) ('Effective' bulk distribution coefficients, defined as $D' =$ (concentration of element in CAPFPM bulk residue) divided by (concentration of element in CAPFPM bulk liquid extract), may differ markedly from D or D^* . In the case of very highly incompatible elements when n is small and z relatively large, D' may be much larger than D , thus reducing greatly the efficiency of the partial melting process in 'stripping' the mantle of its very highly incompatible elements.

(3) By virtue of the [shape factor], concentrations of very highly incompatible elements in CAPFPM products will be

significantly higher in the linear plan case than in simple APFPM products of the same source mantle and higher still in the circular plan case, even if the power factor n remains constant. By virtue of higher thermal losses per unit volume from a circular plan regime than from a linear plan regime, the power factor n is likely to be smaller in the circular plan case than in the linear plan case, enhancing the effect already noted. These effects could be misinterpreted easily as products of mantle heterogeneity, as outlined in the introduction.

(4) The CAPFPM product is much more efficient at fractionating two very highly incompatible elements of similar D^* than is a 'simple' APFPM product a equivalent F or an 'average' value of F (see ref. 14). This effect could reinforce the readily available misinterpretation (see (3) above) that very small mass fractions of partial melting are required to explain the geochemistry of erupted products; or lead to a further misinterpretation that D^* values must be still lower, that is, that mass fractions of trapped melt in the residual mantle must be lower than is actually the case.

(5) There is a disturbing resemblance between the potential misinterpretations of CAPFPM products identified above and the principal conclusions of most publications in the field of igneous geochemistry in the past 15 yr containing interpretations based upon simpler models of the partial melting process. I do not argue here that there are no differences in 'fertility' of different portions of the mantle, or that small mass fractions of partial melt are not, on occasion, extracted, or that large portions of the mantle have not suffered a small fractional melt extraction at some earlier stage of their history. These are probable and readily acceptable conclusions. I do, however, suggest that the geochemical evidence needs to be re-evaluated in order to remove any spurious effects and, thereby, place these conclusions on a more secure foundation.

Alternative assumption integration

Equations (6') and (7') may also be integrated to yield results of the form

$$\left[\begin{array}{c} \text{Complex} \\ \text{aggregated} \\ \text{melt} \end{array} \right] = \left[\begin{array}{c} \text{shape} \\ \text{factor} \end{array} \right] \left[\begin{array}{c} \text{PEPM} \\ \text{at } F_{\max} \end{array} \right] \left[\begin{array}{c} \text{series} \\ \text{factor} \end{array} \right] \quad (10')$$

Shape factors are as before; the PEPM term at F_m is analogous to equation (4') above and the {series factor} in equation (10') is either

$$\{\text{series 3}\} = \left\{ \frac{nn!}{(n+1)!} + \frac{Snn!}{(n+2)!} + \frac{2S^2nn!}{(n+3)!} + \dots + \frac{(p-1)!S^pnn!}{(n+p)!} \right\} \text{—linear case}$$

or

$$[2 \times \{\text{series 3}\} - \{\text{series 4}\}] \text{—circular case}$$

where

$$\{\text{series 4}\} = \left\{ \frac{2n(2n)!}{(2n+1)!} + \frac{S2n(2n)!}{(2n+2)!} + \dots + \frac{(p-1)!S^p2n(2n)!}{(2n+p)!} \right\}$$

and $S = [F_m(1 - D^*)]/[D^* + F_m(1 - D^*)]$.

These relationships differ from those given in equations (8) and (9) above in that they do not contain the subtracted term which produces the strong discrimination between elements of like distribution coefficient. Although it is true that the [PEPM] term produces much the same values of C_L as the [APFPM] term at constant F , it is also true that the [PEPM] term in its own right discriminates much more strongly between highly incompatible elements than does the [APFPM] term. Hence, the previous conclusions (1) (3) and (4) remain valid and (4) is in fact strengthened (see ref. 14). Conclusion (2), however, is not valid as stated. The effective bulk residue–bulk liquid distribution coefficient is D^* . The PEPM process does not strip incompatible elements from the residue with the high efficiency of the APFPM or CAPFPM processes.

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Upper mantle heterogeneities and dynamics

J.-G. Schilling

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881, USA

The width of geochemical and residual elevation anomalies along the Mid-Ocean Ridge System decrease as both the distance between migrating ridge segments and related hotspots and the local geochemical dispersion in the anomalies increase. These observations provide further evidence that flows of mantle plumes in the upper mantle are deflected by migrating ridge sinks once they override hotspots.

THE problem of mantle heterogeneities is related closely to that of mantle dynamics¹. Two extreme models are being pursued currently: one hypothesis considers the dispersion of randomly distributed domains rich in incompatible trace elements, passively embedded in a convecting mantle matrix relatively depleted in such elements and related radiogenic isotopes²⁻⁵. The second considers relatively fixed sources of incompatible element-rich domains, dynamically buoyant, rising as plumes or chains of blobs beneath hotspots which interact and mix with the incompatible element-depleted asthenosphere normally feeding migrating ridges⁶⁻¹⁰. Thus, migrating ridges act as sinks and mantle plumes as sources in this model¹¹.

We have shown recently in Galapagos¹²⁻¹⁴ and South Atlantic¹⁵ regions that ridge segments, once centred on a hotspot but since migrated away as far as 850 km, continue to sense and accrete material with hotspot geochemical signatures. Geochemical anomalies may also have decreased in length, local variability may have increased and the associated ridge elevation anomalies may have decreased as the distance from such ridge segments to nearby hotspots has increased. These observations provide additional evidence for a connecting channel which may develop and extend between the hotspot (the source of large-ion lithophile element (LILE)-rich material) and the migrating ridge (the sink), as proposed initially by Morgan¹¹ and Vink¹⁶ purely on the basis of the tracks left by anomalous constructional volcanism on the sea floor. We also suggested that the flow of plume material towards the migrating ridge-sink becomes discontinuous, perhaps schlieren-like¹⁵.

Here, I extend the testing of this hypothesis by considering more quantitatively how the width of these geochemical and residual elevation anomalies along the ridge crest vary either with distance from migrating ridges to nearby related hotspots, or the time since these ridge segments were located on such hotspots, or current spreading rates. On the basis of these empirical observations and very simple theoretical considerations, I then examine the consequences of this model in predicting the probable geometric pattern and evolution of the connecting flow channels, mixing conditions and constraints on mantle plume fluxes.

Methodology and results

Four major along-ridge geochemical and depth profiles were used as the database (Fig. 1). These include the Galapagos Spreading Centre (83-102° W)¹²⁻¹⁴, the North Atlantic from 79 to 28° N^{17,18}, the South Atlantic from 0° to 47° S¹⁵ and the Gulf of Aden (42-57° E; refs 19, 20 and J.-G.S., unpublished data). The four profiles combined represent about 15,430 km of ridges from the 60,000-70,000-km-long Mid-Ocean Ridge system. The average sample interval is 42 km ± 46 (1 s.d.). These four profiles

comprise 11 recognizable anomalies whose widths are also shown in Fig. 1. The anomaly widths were compiled regardless of whether they represent long regular gradients, as for the ridge-centred hotspots such as Iceland, the Azores and Afar, or irregular spikes with large local dispersion, as for the more distant hotspots such as in the South Atlantic (Fig. 1). Of these 11 anomalies, 9 can be linked readily to a nearby hotspot known or suspected to have influenced the accreting crust at the ridge axis in these regions. Two could not (the 45° N anomaly in the North Atlantic and the short wavelength anomaly in the Gulf of Aden at 45.5° E). These two cases require detailed consideration which will be treated elsewhere with future tests of our model. Table 1 tabulates these geochemical relationships, including their distance to known or suspected hotspots, and the time since these ridge segments were centred on the ridge using plate-reconstructions in the framework of fixed hotspots²¹⁻²⁴. I have also included in Table 1 corresponding current spreading rates taken from ref. 25 and the corresponding maximum residual elevation anomalies above the 2,900-m reference depth of normal ridges suggested by the compilation of Vogt²⁶ and Anderson *et al.*²⁷. Whenever possible, I have attached in Table 1 probable errors associated with these parameters, such as uncertainties related to hotspot locations or plate reconstructions, major fracture zone offsets over the anomalies and sampling coverage and density.

Figure 2 shows that there is a good inverse correlation between the geochemical anomaly-width (W) and the distance (y) from the centre of these anomalies to that of the hotspot suspected or known to be linked to these anomalies (Fig. 2a), or with time (t) since these ridge segments were centred over these hotspots (Fig. 2b). For reference, these two correlations may be expressed by the empirical relations $W = (1,010 - 24.5 (y)^{1/2})$ and $W = (992 - 53.6 (t)^{1/2})$ respectively, with W and y in km and t in Myr, with correlation coefficients r^2 , respectively, 0.85 and 0.89. The somewhat large deviation of the Jan Mayen hotspot from the W versus y reference curve may reflect the uncertainty in the exact location of the ridge axis on the platform and the location of this hotspot. I chose it arbitrarily to be centred on Jan Mayen Island, but this need not necessarily be the case²⁸. The residual ridge-elevation anomaly (ΔE) and associated geochemical anomaly-width also decreases sympathetically (Fig. 3a). A linear relation $\Delta E = -0.34 + 3.13 \times 10^{-3} W$, with ΔE and W in km, can be used as reference (with the Azores point excluded) or $\Delta E = -0.040 + 2.47 \times 10^{-3} W$ (with the Azores point included) with $r^2 = 0.66$ and 0.54, respectively. The two intercepts are, within the uncertainties, close to zero, as expected for normal ridges without geochemical anomalies ($W = 0$) and normal elevation (that is, $\Delta E = 0$, or a reference depth of 2.9 km adopted from ref. 26). The empirical relationship with a linear

Fig. 1 La/Sm database used for determining the width (W) of geochemical anomalies along ridge axes near hotspots (left scales). Sources of profiles are: Mid-Atlantic Ridge profiles 78–71° N, 71–28° N and 0–47° S from refs 18, 17 and 15, respectively; Galapagos 102–82° W from ref. 12; Gulf of Aden 42–57° E (from J.-S.G., manuscript in preparation). La/Sm_N is the La/Sm ratio normalized to chondrites; solid-line profiles represent the depth of sample recovery on the ridge axis, smoothed out with a method of moving average of ± 100 km from each station. These depth profiles approximate the elevation of the ridge axis (see Table 1 for more exact values).

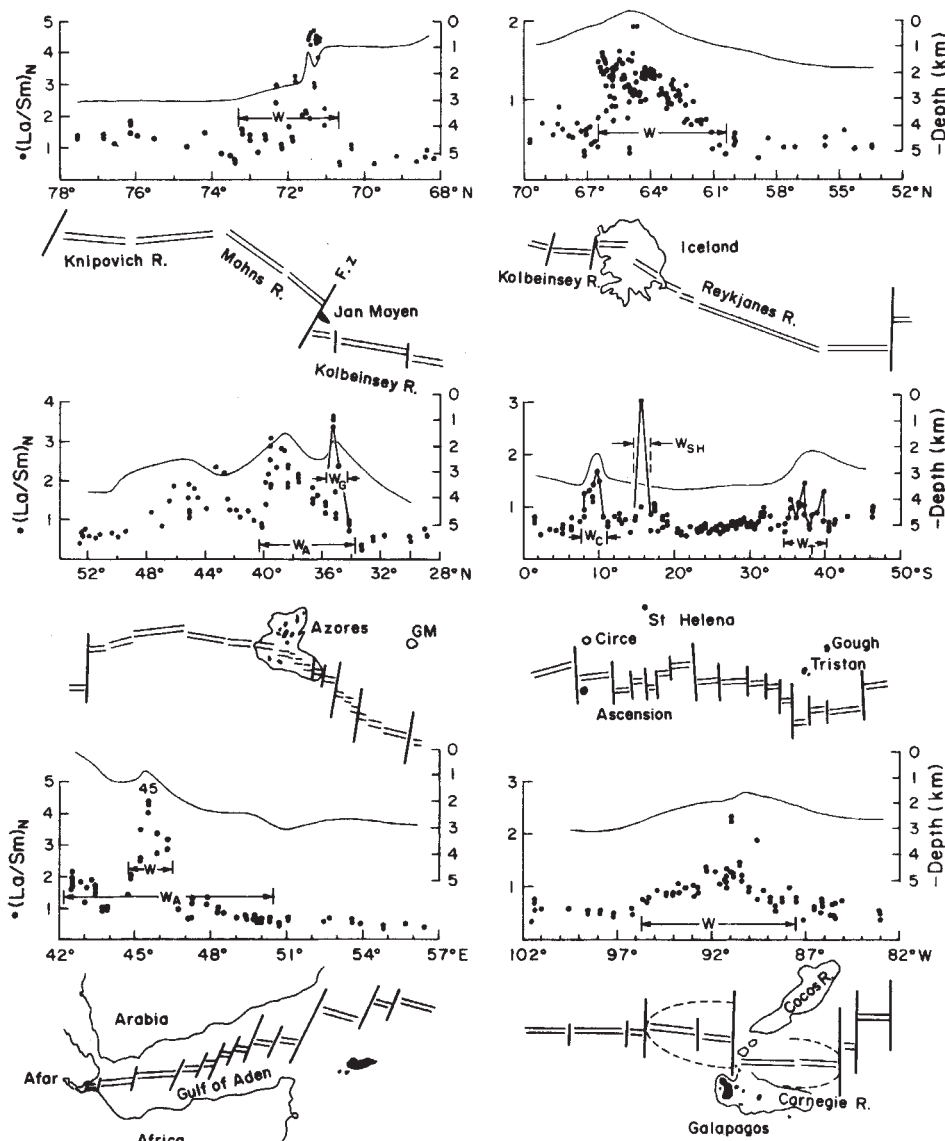


Table 1 Data used to construct Figs 2 and 3

Ridge anomaly	Associated hotspot	Hotspot location	Hotspot symbol*	Anomaly width (W) (km)	Residual† elevation (ΔE) (km)	Distance hotspot to anomaly (y) (km)	Overriding time (Myr)	Full spreading rate (S) (cm yr ⁻¹)
North Atlantic								
71°–73° N	Jan Mayen	71°10' N, 8°0' W	JM	668 ± 115	2.2 ± 0.2	75	—	1.66
60°–66° N	Iceland	65°0' N, 19°0' W	IC	923 ± 210	3.4 ± 0.2	0	0	1.90
41°–50° B	? (45° N)	—	—	975 ± 62	0.8 ± 0.3	—	—	2.37
33°–40° N	Azores	39°38' N, 29°45' W	AZ	1,904 ± 100	1.8 ± 0.3	0	0	2.46
34°–36° N	Great Meteor	28°0' N, 33°0' W, 29°0' N, 29°0' W	GM	323 ± 100	1.1 ± 0.2	835–897	70	2.38
South Atlantic								
7°–11° S	Circe (Ascension)	8°15' S, 9°20' W	C	393 ± 108	1.2 ± 0.2	444	190	3.87
15°–16° S	St Helena	16°0' S, 5°45' W	SH	208 ± 96	0 ± 0.3	811	190	3.93
35°–40° S	Tristan	37°15' S, 12°18' W	T	565 ± 100	0.7 ± 0.1	388–444	80	3.85
Red Sea/Gulf of Aden								
42°–51° E	Afar	11°40' N, 42°30' E	AF	1,000 ± 145	2.9 ± 0.2	0	0	1.80–2.02
45°–46° E	? (45.5° E)	—	—	159 ± 45	2.1 ± 0.2	—	—	1.88
Equatorial Pacific								
95°–87° W	Galapagos	0°42' S, 91°30' W	G	880 ± 124	1.3 ± 0.1	213–296	7.5	6.02

* Used in Figs 2, 3. † Maximum residual elevation of geochemically anomalous ridge segment over and above a normal depth of 2.9 km adopted from ref. 26, using data from ref. 26 and modified from my own observations during dredging.

best fit forced through the origin and the Azores point included is $\Delta E = 2.42 \times 10^{-3} W$, with $r^2 = 0.87$. Vogt²⁶ has shown that the residual ridge anomaly located on or near hotspots is inversely related to spreading rates. Figure 3b shows that the residual ridge elevation anomaly is also related to the distance of these anomalous ridge segments to their corresponding hotspot. A possible empirical relationship is $\Delta E = 2.7 - 7.7 \times 10^{-2}(y)^{1/2}$ or $\Delta E = 3.0 - 9.2 \times 10^{-2}(y)^{1/2}$, with ΔE and y in km and r^2 of 0.75 and 0.87, with or without the Azores point, respectively. The possible departure of the Azores hotspot from the correlation involving the residual ridge elevation anomaly may be related to the fact that this hotspot has become ridge-centred to its present position only 9 Myr ago, judging from kinematic plate reconstructions²¹⁻²⁴, as well as geochemical results from the IPOD Leg 82 sampling grid in the region²⁹. The Mid-Atlantic Ridge segment just north of the Azores passed over this hotspot once before, ~38 Myr ago²¹⁻²⁵. Finally, I note that there is apparently no correlation between the anomaly-width and present spreading rates, although the range of spreading rate is limited mostly to 0.8–2 cm yr⁻¹ half-rate, with one exception (Galapagos, 2.8–3.1 cm yr⁻¹) (Fig. 2c).

The above correlations suggest that as the ridge migrates away from the hotspot, both the width of the geochemical anomaly and associated anomalous elevation caused by the hotspot decrease progressively with time. The spreading rate does not seem significantly influential, at least over the small range so far investigated.

These regularities suggest strongly that some connection exists between hotspots and migrating ridge segments which are anomalous geochemically and in residual elevation. This evidence provides further support for the development of a lateral flow, maintained between these migrating ridge sinks and corresponding mantle plumes, since the time when these ridges were initially centred on these hotspots¹⁵.

Modelling

Ridge-centred hotspot case. We have proposed previously that along-ridge geochemical gradients about ridge-centred hotspots can be modelled by a binary mixing model comprising as end members the LILE-rich blobs and the LILE-depleted asthenosphere⁷⁻¹⁰. The model requires that the blob delivers material at a rate independent and in excess of the rate of accretion by sea-floor spreading over the blob; the plume tends to elevate the region. The model also assumes that in the absence of a plume, accretion is passive and results simply from the divergence of the plates. The excess of blob material tends to pile up and form thicker crusts and is also accommodated by some flow outward along the ridge which acts as a sink. The mixing proportion (P) of blob material relative to the total mass of material accreting per unit ridge-length could decrease from unity over the plume (providing that no dilution with the depleted asthenosphere took place while travelling through it, or that the melting-zone width across the ridge is smaller than that of the blob) to zero at the end of the gradient where plume material has been completely used up by the accretion process (Fig. 4). We have shown previously that by estimating the trace-element content and the isotopic composition of the two end members (or Sm content and La/Sm ratios), the variation of P along the ridge axis (taken as the x direction) can be determined readily from the isotopic ratio variation (or La/Sm; see refs 12, 13). We can also estimate the minimum flux of the blob (Q), assuming that the gradient in La/Sm represents a quasi-steady state, namely:

$$Q = 2 \int_{x=0}^L H(x) S(x) P(x) dx \quad (1)$$

Observations suggest that the crustal thickness (H) and the full spreading rate (S) may both be functions of the distance (x) from the blob. In the evaluation of the physics of the process, it is probable that $H(x)$ would be dependent on $S(x)$. To illustrate the point simply, however, I assume in Fig. 4 that: (1) the blob is a point source at $x=0$; (2) the two gradients are

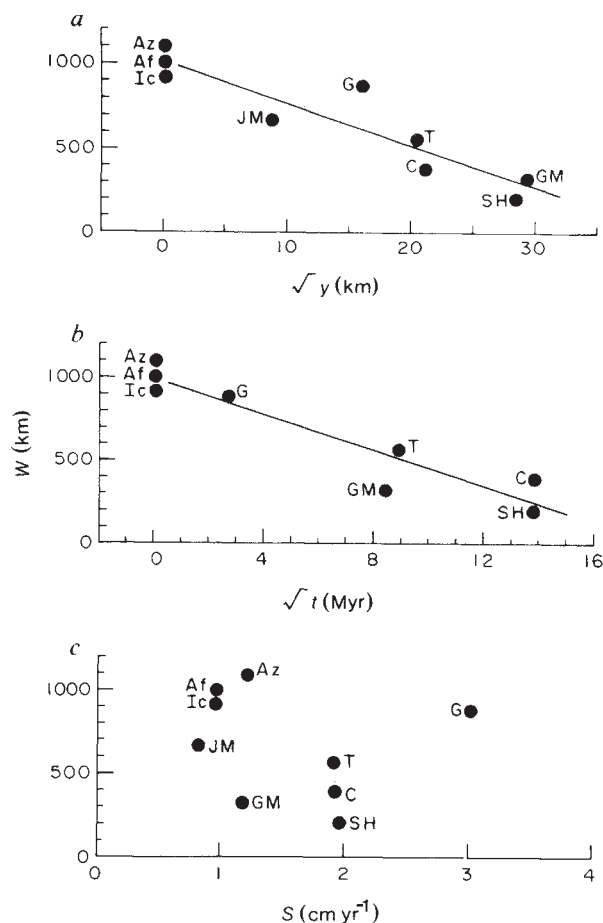


Fig. 2 Geochemical anomaly width (W) versus: *a*, square root of present distance (y) of associated hotspot to the geochemical anomaly on migrating ridge (determined from Fig. 1); *b*, square root of time (t) since the ridge was centred on associated hotspot (determined from refs 22–24); and *c*, half spreading rates (S) (determined from ref. 25). All values used, including symbols used for hotspot names, are listed in Table 1. Note the good best-fit inverse linear correlation of W with $y^{1/2}$ and W with $t^{1/2}$ with correlation coefficients r^2 of 0.85 and 0.89 respectively, but lack of correlation of W with S .

placed symmetrically about the blob at $x=0$, hence the factor two in equation (1); (3) the two end-member materials have essentially the same density, thus $P(x)$ represents volumetric proportions and Q is in km³ yr⁻¹, if S is in km yr⁻¹; (4) H and S are constants independent of (x); (5) P varies linearly with x and is given by $P = 1 - (x/L)$, with ($0 \leq P \leq 1$; Fig. 4). Under these conditions, integration of equation (1) gives

$$Q = HSL = HSW/2 \quad (2)$$

or

$$W = 2Q/HS \quad (3)$$

where W is twice the length of gradients distributed symmetrically about the blob.

Thus, for ridge-centred blobs $W = 2Q/HS$ and is directly proportional to the blob flux and inversely proportional to S and H . For a given plume flux and constant crustal thickness, W or the length of the gradient L , should decrease with increasing spreading rate from one ridge-centred hotspot to another.

In reality, equation (1) must be integrated separately for each ridge-centred hotspot situation, with different boundary conditions and appropriate $H(x)$, $S(x)$ and $P(x)$ functions. In such cases, the relation of Q with L or W may be of a higher order than indicated in equations (2) or (3). Minimum estimates of plume fluxes can be made by this method and will be reported on in detail elsewhere.

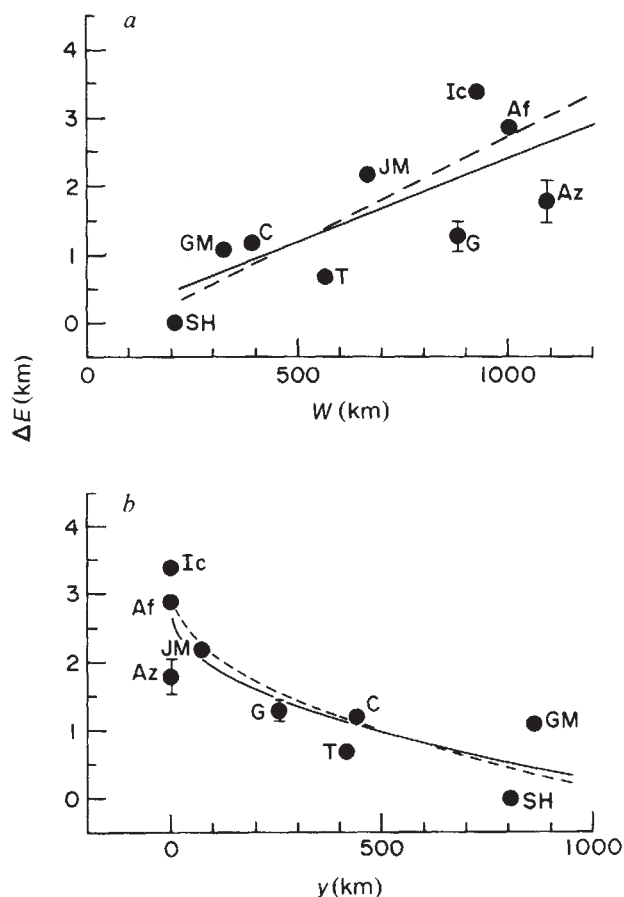


Fig. 3 Residual elevation (ΔE) of ridge axis over geochemical anomalies versus: *a*, geochemical anomaly width W (solid and dashed lines are linear best-fits including and excluding the Azores, respectively and the correlation coefficients are respectively, 0.54 and 0.66); *b*, distance (y) of hotspot to the geochemical anomaly on the migrating ridge (dashed and solid curves are fitted to data with and without the Azores respectively; see text for empirical relationships). Residual elevation ΔE is the maximum elevation observed along the geochemical anomaly above the 2,900 m-depth base line of normal ridge axes adopted from ref. 26 (that is, ridge segments not influenced by hotspots and without any geochemical anomalies). All values used are listed in Table 1.

Finally, I note that Vogt²⁶ has modelled independently fluxes for along-ridge subcrustal pipe flows from hotspots on the basis of topographic gradients. These fluxes²⁶ should not be confused with the flux in my equation (1) which refers only to material with plume signature. The latter could be only a fraction of the total flux taking place along the ridge proposed by Vogt's model²⁶.

Off-ridge hotspot case. I can now extend the steady-state, ridge-centred hotspot model to the situation where there are fixed off-ridge hotspot sources but migrating ridges. Here the flux Q represents the rate at which plume material is being discharged at the mouth of the lateral channel connecting the off-ridge hotspot to the migrating ridge. I refer to this discharge flux as Q_d . Any excess of plume-derived material would be deflected along the ridge axes on either side of this intersection and be used up progressively by the accreting process. As I have determined empirically that W is related inversely to the distance of the ridge to the hotspot (y) (or inversely related to the time (t) elapsed since the migrating ridge was over the hotspot), it follows from equation (3) that the Q_d of plume-related material reaching the migrating ridge may, to a first approximation, be related inversely to the distance y ($Q_d \propto 1/y$) (or inversely related to t). In other words, the amount of plume material (or mixture of plume and asthenospheric materials) reaching the migrating

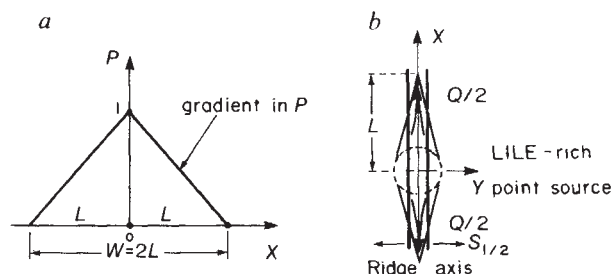


Fig. 4 *a*, Steady state, subcrustal ridge-centred mantle-plume flow model considered in text. Diamond-like figure delineates idealized boundary of plume flow which along x feeds in part of the spreading ridge axis (heavy parallel line) with LILE-rich material. *b*, Corresponding variation with x of the fraction (P) of LILE-rich plume material entering the mix with LILE-depleted asthenosphere to form the new oceanic lithosphere. The two vectors represent integrated plume flux $Q/2$ along the x direction when a steady state is reached with new lithosphere formation by spreading along the ridge axis (sink).

ridge should decrease progressively with time and as the migrating ridge becomes more distant from the hotspot, until it becomes essentially intraplate. Eventually, our steady-state model will no longer apply because of the lack of excess plume flow; only a trickling of plume material will reach the ridge with very inefficient mixing¹⁵. Here, (for example, St Helena) W may only approximate the width of the lateral connecting channel. Of course, I do not suggest that by combining all the hotspot-migrating ridges so far studied, an inverse relationship should exist between the plume discharge rates Q_d and y or t . This stems from the fact that the blob size and total plume flux Q may vary from one hotspot to another, or with time for a single hotspot. As I have noted previously, the spreading-rate variation with x , which may also affect $H(x)$, will differ from one hotspot-ridge system to another.

Flow pattern

Two simple types of arguments provide additional support for suggesting in plan form in the upper mantle a relatively narrow flow channel from the off-ridge hotspot to the migrating ridge.

The first method is based on the comparison of the spatial geochemical variation and derived P values observed with those predicted in plan form from a radially zoned mantle-plume flow tapped passively by the migrating ridge (Fig. 5a). This approach is particularly amenable to tests if large fracture zone offsets are present, for example, the Galapagos¹²⁻¹³, thus causing major discontinuities in distances from the ridge axis to the hotspot and predicting discontinuities in the geochemical variations and related P values. Here, it was shown geochemically that such a radially zoned plume flow model is not viable; instead, a northward channelled plume flow toward the ridge is more appropriate, as suggested independently by Morgan¹¹ on the basis of constructional volcanism and plate kinematics. The same kind of argument can be used for the Tristan configuration, where the Mid-Atlantic Ridge stations located nearest to the Tristan hotspot are not the most enriched in La/Sm (Fig. 1). This test, however, becomes more difficult to apply to the case of the most distant St Helena, Circe and Great Meteor hotspots, because of the large local dispersion and poor mixing conditions observed in the related along-ridge geochemical anomalies and the lack of major ridge offsets along these segments.

In the second type of test, I consider only the variation of W with t or y to the hotspot, regardless of the form of the variation $P(x)$ in the anomaly (Fig. 5b, c). Figure 5b shows again a model which considers in plan form an outward radial plume flow, extending to a radius R and its passive tapping by the migrating ridge above. The radial variation of the P value need not be specified. The circumference limit at R can be considered physically either as the location where the plume is so diluted with

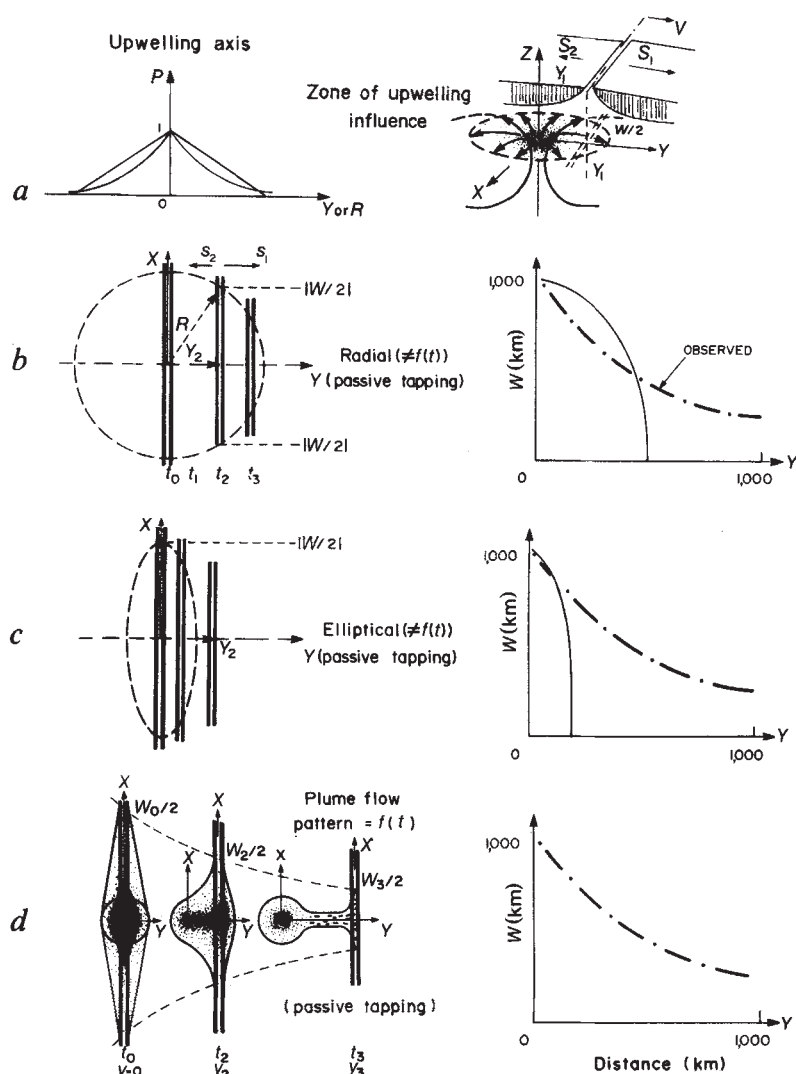


Fig. 5 Models of plume-flow (source) migrating ridge (sink): The vectors S_1 and S_2 are the half-spreading rates relative to the ridge axis; V is the component of the absolute motion of the migrating ridge along the spreading direction and relative to the hotspot which is considered fixed in space. The mixing fraction P of LILE-rich material is specified only in model *a*, where it decreases regularly outward from the centre of the plume. In model *b*, the zone of plume influence detected and passively tapped by the migrating ridge is circular and independent of time, whereas in *c* it is elliptical. Both models *a* and *b* predict a variation of W on the ridge with y which is concave downward, instead of upward as observed empirically in Fig. 2. Model *d* considers a plume-flow pattern variable in time and influenced by the migrating ridge, hence changing pressure gradients. The plume-flow configuration is shown for three different times. At t_0 , the plume is ridge-centred, the ridge is elevated anomalously, the plume flow is mostly along the x -axis, and P varies regularly with x (same model as in Fig. 4, for example, Iceland). At time t_3 , the plume flow is now channelled toward the more distant ridge, W on the ridge is minimal and the elevation is now essentially normal, mixing is irregular and the heterogeneities are schlieren-like (for example, St Helena-Mid Atlantic Ridge). At time t_2 , an intermediate case, the plume flow is skewed both toward and along the migrating ridge which acts as a sink (for example, Galapagos).

the depleted asthenosphere that it can no longer be detected or that the radial plume panache is returning down into the mantle and at the radius R the return flow is deeper than the partial melting zone where lavas used in the sampling take source (Fig. 5b). As the ridge is migrating along the y direction over the zone of influence of the plume (that is, when $y < R$), the length of the ridge over which material with plume signature can be detected (that is, W) decreases as expected from the empirical relations established in Fig. 2. The predicted form of the variation of W with y , however, is concave downward, rather than upward as actually observed. This is also the case if the zone of upwelling plume influence is elliptical as a result of initial down-flow along the ridge when it was centred over the hotspot (Fig. 5c). These two radial models, where the shape of the zone of plume influence in the upper mantle is considered to be essentially constant and independent of time, must therefore be rejected. A more dynamic situation is required to match the empirical variation of W with t or y (Fig. 5d). The plume-flow pattern about the hotspot must change with t as the plume-source/ridge-sink geometric configuration and associated resulting pressure gradients change progressively from ridge-centred to a near-ridge and finally to completely off-ridge.

We believe that the model of a highly skewed plume flow along the ridge when the hotspot was ridge-centred and the lateral flow which developed subsequently and continued to feed the plume flow along the ridge as it begins to migrate away from the hotspot, can explain satisfactorily the observed variation of W with t or y ; the model is sketched in Fig. 5d. As long as the discharge rate at the mouth of the channel exceeds that of the accretion process per unit ridge length at this intersec-

tion, some flow will take place along the ridge and a gradient may develop if mixing conditions are regular. As the distance of the ridge to the hotspot increases with t , the plume flow along the lateral channel and the ridge axis decreases as does the width of the anomaly. The ridge crest also subsides and the mixing conditions become more irregular; considerable dilution with the surrounding depleted asthenosphere seems to occur along the channel, perhaps by entrainment and shearing associated with return flows toward the ridge in the upper mantle. Figure 5d illustrates how W may change with time, assuming that the empirical relationship observed from the pool of ridge-hotspot configuration so far studied is directly applicable to a single hotspot-ridge situation. W would decrease rapidly as soon as the migrating ridge leaves the hotspot and the rate of decrease slows down as y increases.

To conclude, I emphasize that, in general, the regularities observed between the geochemical and elevation anomalies along the ridge crest and their position relative to hotspots are clearly not random and are probably not coincidental. Thus, I believe that the model of hotspot-source migrating-ridge sink and a connecting flow channel deserves further consideration and testing. The regularities noted should also be taken into consideration by proponents of the alternative stochastic plume-pudding model^{4,5}. With these modifications, these two extreme models need not be necessarily mutually exclusive.

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Hypervariable 'minisatellite' regions in human DNA

Alec J. Jeffreys*, Victoria Wilson* & Swee Lay Thein†

* Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK

† MRC Molecular Haematology Unit, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK

The human genome contains many dispersed tandem-repetitive 'minisatellite' regions detected via a shared 10–15-base pair 'core' sequence similar to the generalized recombination signal (χ) of Escherichia coli. Many minisatellites are highly polymorphic due to allelic variation in repeat copy number in the minisatellite. A probe based on a tandem-repeat of the core sequence can detect many highly variable loci simultaneously and can provide an individual-specific DNA 'fingerprint' of general use in human genetic analysis.

DNA POLYMORPHISMS have revolutionized human genetic analysis and have found general use in antenatal diagnosis¹, mapping of human linkage groups^{2,3}, indirect localization of genetic disease loci by linkage^{2,4,5} and analysis of the role of mitotic nondisjunction and recombination in inherited cancer^{6–9}. Single-copy human DNA probes are used normally to detect restriction fragment length polymorphisms (RFLPs), most of which result from small-scale changes in DNA, usually base substitutions, which create or destroy specific restriction endonuclease cleavage sites^{10,11}. As the mean heterozygosity of human DNA is low (~0.001 per base pair)^{10–12}, few if any restriction endonucleases will detect a RFLP at a given locus, although the probability of detection is improved for enzymes such as *MspI* and *TaqYI* which contain the mutable CpG doublet in their recognition sequence¹³. Even when detected, most RFLPs are only dimorphic (presence or absence of a restriction endonuclease cleavage site) with a heterozygosity, determined by allele frequencies, which can never exceed 50% and which is usually much less. As a result, all such RFLPs will be uninformative in pedigree analysis whenever critical individuals are homozygous.

Genetic analysis in man could be simplified considerably by the availability of probes for hypervariable regions of human DNA showing multiallelic variation and correspondingly high heterozygosities. The first such region was isolated by chance by Wyman and White¹⁴ from a library of random segments of human DNA. The structural basis for multiallelic variation at this locus is not yet known. Subsequently, and again by chance, several other highly variable regions have been discovered near the human insulin gene¹⁵, α -related globin genes^{16–18} and the c-Ha-ras-1 oncogene¹⁹. In each case, the variable region consists of tandem repeats of a short sequence (or 'minisatellite') and polymorphism results from allelic differences in the number of repeats, arising presumably by mitotic or meiotic unequal exchanges or by DNA slippage during replication. The resulting minisatellite length variation can be detected using any restriction endonuclease which does not cleave the repeat unit and provides for such loci a set of stably inherited genetic markers.

We have described previously a short minisatellite comprised of four tandem repeats of a 33-base pair (bp) sequence in an

intron of the human myoglobin gene (ref. 20; Fig. 1). The 33-bp repeat showed some similarity in sequence to three other hypervariable human minisatellites characterized previously and on the basis that the myoglobin minisatellite was flanked by a 9-bp direct repeat characteristic of the target site duplications generated by transposable elements, we suggested that this minisatellite and some other hypervariable regions were related via transposition. We show here that the myoglobin 33-bp repeat is indeed capable of detecting other human minisatellites, some of which are highly polymorphic. These regions, however, are not related by transposition, but instead share a common short 'core' sequence in each repeat unit, which in turn provides a powerful probe for hypervariable regions.

Probe for variable human DNA

A pure repeat probe was prepared from the human myoglobin minisatellite by purification of a single 33-bp repeat element followed by head-to-tail ligation and cloning of the resulting polymer into pUC 13 (ref. 21; Fig. 1). Cleavage of one of the resulting recombinants, pAV33.7, with *BamHI* plus *EcoRI* released a 767-bp DNA insert comprised almost entirely of 23 repeats of the 33-bp sequence.

Low stringency hybridization of this repetitive insert to human DNA digested with restriction endonuclease *EcoRI* detected numerous cross-hybridizing DNA fragments, some of which showed signs of polymorphic variation (data not shown). To improve the detection of polymorphisms, the hybridization was repeated with human DNA digested with *HinfI* or *HaeIII*, both of which cleave at a 4-bp sequence not present in the 33-bp repeat sequence and which should release minisatellites in relatively small DNA fragments whose size will reflect more closely the number of repeats per minisatellite. As shown in Fig. 2, the repetitive probe detected multiple DNA fragments in human DNA as well as the parent DNA fragment from the human myoglobin gene. The larger DNA fragments (in the range 2–6 kilobases (kb) and substantially larger than the mean DNA fragment size of ~0.3 kb in human DNA digested with *HinfI* or *HaeIII*) in particular showed variation between the three individuals examined; these variants were transmitted apparently in a Mendelian fashion, in that each polymorphic

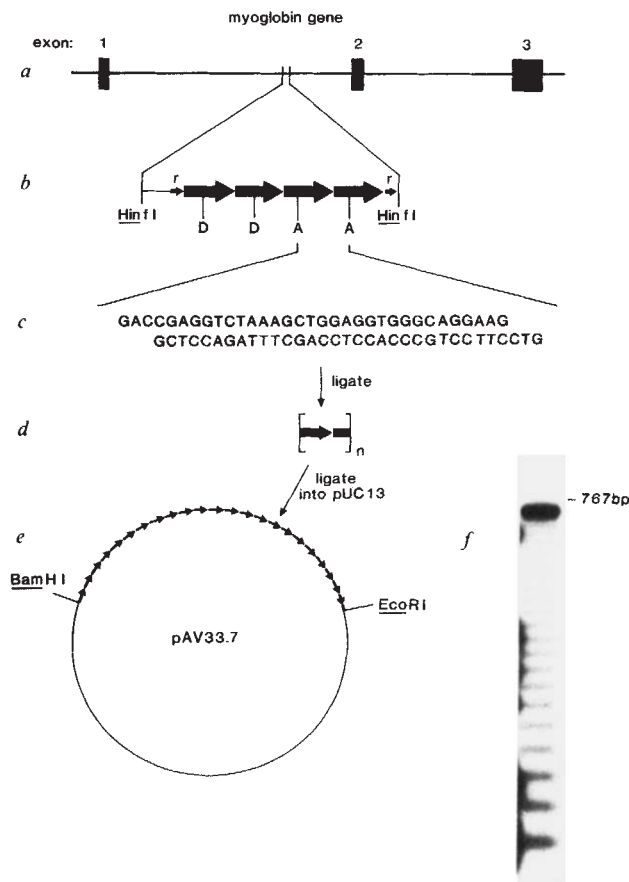


Fig. 1 Construction of a tandem-repetitive hybridization probe for 33-related DNA sequences. *a, b*, This probe was derived from a tandem repetitive segment of the human myoglobin gene²⁰. This region, located in the first intron and comprising four repeats of a 33-bp sequence flanked by a 9-bp direct repeat (r), was isolated in a 169-bp *HinfI* fragment, end-repaired and amplified by cloning into the *SmaI* site of pUC13 (ref. 21). *c*, A 33-bp repeat monomer was isolated by cleaving the third and fourth repeat with *AvaII* (A); a single base substitution in repeats 1 and 2 eliminates this site and creates instead a *DdeI* (D) cleavage site²⁰. *d*, Ligation of the 33-bp monomer via the non-identical *AvaII* sticky ends produced a head-to-tail polymer. Polymers containing ≥ 10 repeats were isolated by preparative agarose gel electrophoresis³³, end-repaired, ligated into the *SmaI* site of pUC13 and cloned in *E. coli* JM83 (ref. 21). *e*, The structure of clone pAV33.7 was confirmed by excision of the insert at the polylinker with *BamHI* plus *EcoRI*, fill-in labelling with α -³²P-dCTP at the *BamHI* site, and partial digestion with *AvaII*. *f*, Labelled partial digest products were resolved by electrophoresis on a 2% agarose gel. pAV33.7 contains 23 repeats of the 33-bp monomer contained in a 767-bp *BamHI/EcoRI* fragment as shown (*e*).

band in the daughter could be identified within one or other (but not both) parents. Variation was detectable in both *HinfI* and *HaeIII* digests of human DNA, consistent with polymorphism resulting from length variation of minisatellite regions.

Isolation of minisatellites

A human genomic library²⁰ of 10–20 kb *Sau3A* partials of human DNA cloned in phage λ L47.1 (ref. 22) was screened by hybridization with the 33-bp repeat probe from pAV33.7. At least 40 strongly-to-weakly hybridizing plaques were identified in a library of 3×10^5 recombinants, consistent with the complexity of the Southern blot hybridization (Fig. 2). A random selection of eight of these positive plaques was purified (λ 33.1–15) and Southern blot analysis of phage DNA showed that in each recombinant the hybridizing DNA was localized in a unique short (0.2–2 kb) region of the recombinant. Sequence analysis (Fig. 3) showed that this region in each of the eight recombinants

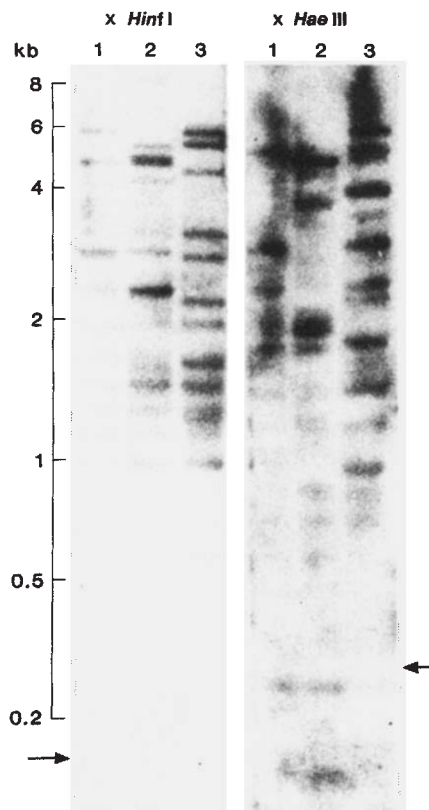


Fig. 2 Detection of multiple 33-related sequences in human DNA. 10 μ g samples of DNA from individual 1 (daughter), 2 (mother) and 3 (father) were digested with *HinfI* or *HaeIII*, electrophoresed through a 1% agarose gel and transferred by blotting³⁵ to a Pall Biodyne membrane to prevent the loss of small DNA fragments. The membrane was hybridized in $1 \times$ SSC at 60° with dextran sulphate³⁶ to the insert from pAV33.7 (Fig. 1) labelled *in vitro* with ³²P (ref. 20). The arrowed fragment in each digest is derived from the minisatellite located in the myoglobin gene (Fig. 1); a survey of DNA from 12 individuals digested with *HaeIII* showed that this myoglobin minisatellite is monomorphic (data not shown).

contains a minisatellite of 3–29 tandem copies of a repeat sequence whose length ranged from 16 bp in λ 33.15 to 64 bp in λ 33.4. Most minisatellites contained an integral number of repeats. In λ 33.6, the 37-bp repeat consisted in turn of a diverged trimer of a basic 12-bp unit. Each λ 33 recombinant represented a different region of the human genome, judged by the clone-specific DNA sequences flanking each minisatellite.

Highly polymorphic minisatellites

The eight cloned minisatellite regions were located in 0.5–2.2-kb *HinfI* DNA fragments, smaller than the clearly polymorphic 2–6-kb DNA fragments detected by pAV33.7 in *HinfI* digests of human DNA (Fig. 2). To determine whether any of the cloned regions were also polymorphic, ³²P-labelled single-stranded DNA probes were prepared from suitable M13 subclones of each minisatellite and hybridized at high stringency to a panel of 14 unrelated British caucasian DNAs digested with *HinfI*. Typical hybridization patterns are shown in Fig. 5, showing that under these hybridization conditions each probe detects a unique region of the human genome. Alleles detected by each probe are summarized in Table 1 where in each case, the most common *HinfI* allele corresponded in size to the *HinfI* minisatellite fragment in clones λ 33.1–15, suggesting that these regions have been isolated without major rearrangement.

In the limited population sample studied, four of the eight minisatellites showed polymorphic variation. Three of the regions were highly polymorphic with between five and eight resolvable *HinfI* fragment-length alleles detected per locus. This variation almost certainly results from variation in the repeat

Table 1 Allelic variation at individual cloned minisatellites

Clone $\lambda 33$	Repeat length (bp)	Divergence %	Alleles			$4N_e u$		
			Length (bp)	No. repeats	Frequency	A	B	C
1	62	0.2 ± 0.2	3,150	40	0.04	2	3	9
			2,600	31	0.11			
			2,350	27	0.04			
			*2,300	26	0.71			
			2,190	24	0.07			
			1,950	20	0.04			
3	32	14.1 ± 2.5	*450	6	1.00	0	0	0
4	64	6.9 ± 1.5	2,280	18	0.07	2	2	5
			2,140	16	0.11			
			*2,015	14	0.43			
			1,950	13	0.36			
			1,780	10	0.04			
5	17	9.2 ± 1.9	*1,660	14	1.00	0	0	0
6	37	0.7 ± 0.4	1,800	25†	0.04	4	6	20
			1,650	21†	0.07			
			1,570	19†	0.04			
			*1,535	18†	0.43			
			1,450	16†	0.04			
			1,400	15†	0.25			
			1,350	14†	0.04			
			1,280	12†	0.11			
10	41	5.9 ± 0.6	*1,460	5	1.00	0	0	0
11	33	0.0 ± 0.0	*990	3	1.00	0	0	0
15	16	1.1 ± 0.5	1,410	41	0.50	0.3	0.3	0.3
			*1,220	29	0.50			

DNA was prepared from white blood cells¹⁰ from 14 unrelated British caucasians (seven male, seven female), digested with *Hin*II and Southern blot hybridized at very high stringency with probes from subcloned minisatellites as described in Fig. 5a, b. Cloned alleles whose sequences are shown in Fig. 3 are indicated (*). The divergence of each sequenced minisatellite from a hypothetical (consensus)_n sequence is given as the mean percentage unique substitution divergence ($\pm$ s.e.m.) to correct for variants which have diffused over more than one repeat. For example, $\lambda 33.15$ shows 13 variants (10 substitutions and three deletions) but only five distinct variants over 29 repeats of a 16-bp sequence, giving a divergence from a (consensus)₂₉ sequence of $5/(29 \times 16) = 1.1\%$. The number of repeats in each allele was estimated from DNA fragment size; this estimate for alleles of $\lambda 33.6$ is approximate (†) because of the trimeric nature of the $\lambda 33.6$ repeat unit (Fig. 3). Approximate values of $\theta = 4N_e u$, where N_e is the effective population size and u is the rate of production of new length variants per locus per gamete, were estimated for each minisatellite from the number of different alleles (n_a) in the sample of 14 individuals by extensive population computer simulations designed to estimate the number of different, selectively equivalent alleles that could be maintained at steady-state in a population according to three recombinational models: A, random unequal crossing over between two alleles at meiosis, giving a new allele comprised of a random-length 5' segment of one allele fused to random length 3' segment of the second allele; B, constrained unequal exchange such that an allele mutates at random to gain or lose between one and three repeats; C, constrained slippage causing the gain or loss of only a single repeat. In each model, it is assumed as a first approximation that u is independent of the number of repeats in allele, as for the loci presented there is at most only a two-fold variation in allele length. Simulations were performed for values of θ from 0.1 to 100, using population sizes N_e from 50 to 500, and were continued for 10 N_e generations, steady state being achieved within $\sim N_e$ generations. Results from model A closely approximated the infinite allele model at $\theta < 2$, when the expected number of alleles n_a in a sample of i individuals is given by $n_a = \sum_{i=1}^{2i} (\theta/\theta + i - 1)$ (ref. 31). Model C is the charge state model for which n_a has yet to be solved as a function of θ (ref. 32).

copy number in minisatellites, as alleles generally differed in length by an integral number of repeat units. In addition, longer alleles tended to hybridize with minisatellite probes more strongly than shorter alleles (see individual 1 in Fig. 5a), again suggesting that longer alleles contain more repeat sequence.

There is considerable variation in the level of repeat sequence homogeneity in each sequenced minisatellite region (Fig. 3). Some minisatellites (for example, $\lambda 33.3$ and 5) show substantial repeat divergence, suggesting that these regions are not actively undergoing sequence homogenization by unequal exchange²³; as expected, these regions show no polymorphic variation in repeat number (Table 1). Instead, the highly polymorphic minisatellites ($\lambda 33.1$, 4 and 6) all show high repeat copy number together with substantial sequence homogeneity of repeats. In addition, base substitutions in the repeat units of hypervariable minisatellites tend to be present in more than one repeat (see, for example, $\lambda 33.15$ in Fig. 3) which indicates that these minisatellites are actively and repeatedly engaging in unequal exchange, resulting in the diffusion of novel base substitutions across more than one repeat unit²³.

We used computer simulations to estimate the rate of unequal exchange needed to maintain the number of different (neutral) alleles (n_a) seen in our population sample (Table 1). Although space does not allow a detailed description of the population

simulations, we find that for a population of N_e diploid individuals starting with a monomorphic minisatellite containing say 30 repeats, n_a reaches a steady state in $\sim N_e$ generations, the mean value of n_a being determined both by the parameter $\theta = 4N_e u$, where u is the rate of production of new length alleles per locus per gamete, and by the model of unequal exchange used in the population simulation. Three models have been investigated: (1) random meiotic unequal exchange between minisatellite alleles; (2) constrained sister chromatid exchange; (3) DNA slippage causing the gain or loss of a single repeat (Table 1, models A, B and C respectively). We favour the constrained exchange model because of the tendency for minisatellite base substitution variants to diffuse to non-adjacent repeats (Fig. 3), together with the tendency of different length alleles of minisatellites to differ from each other by several rather than either one or many repeat units (Table 1). For the highly polymorphic minisatellites, we estimate θ to be 2–6. Given that the effective population size N_e for human populations has been estimated at $\sim 10^4$ (ref. 24), this gives values of u , the mutation rate to a new length allele, of $\sim 0.5\text{--}1.5 \times 10^{-4}$ per gamete for a minisatellite ~ 1 kb long.

This value is higher than the base substitution neutral mutation rate in man, which from studies of non-coding DNA sequence divergence in man and higher primates has been

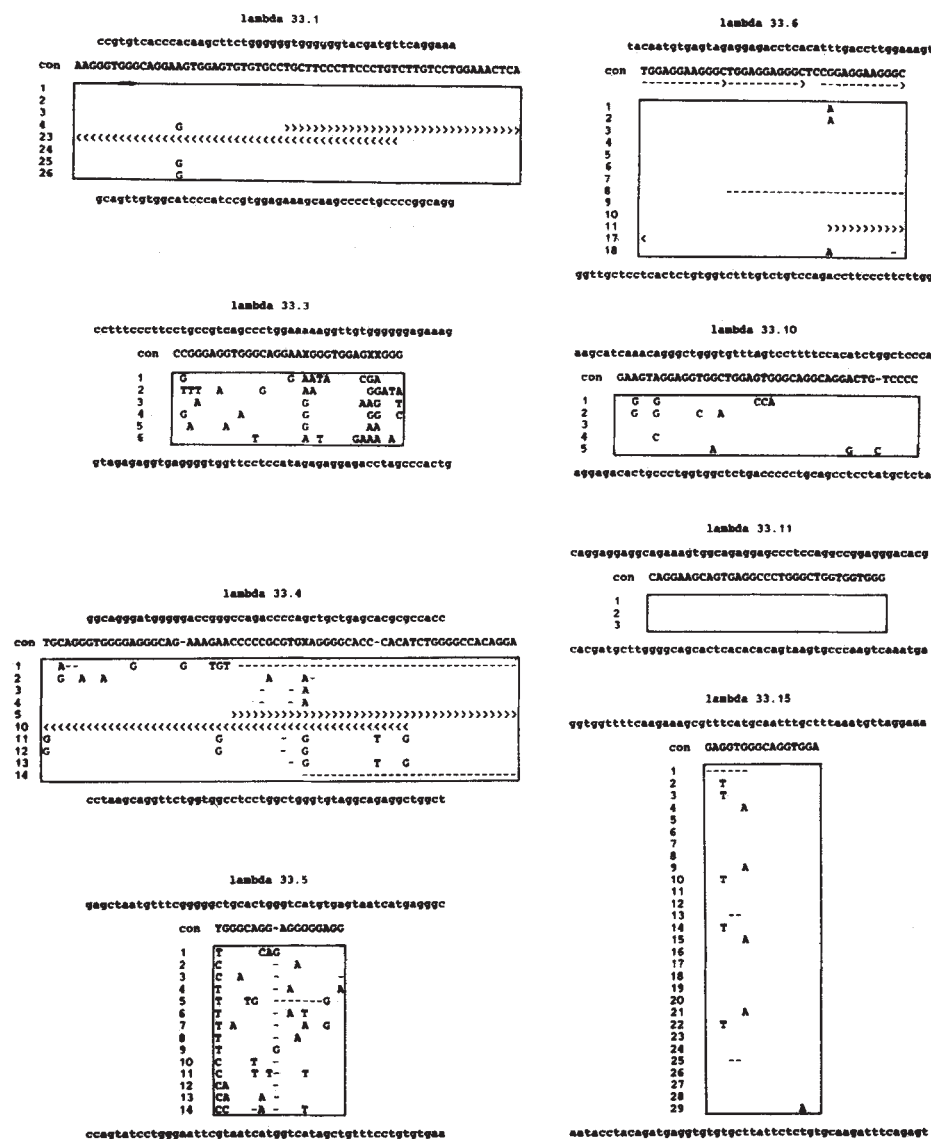


Fig. 3 Sequences of a selection of minisatellite regions detected by the myoglobin 33-repeat probe. The consensus sequence (con) of the tandem repetitive region in each of the genomic clones λ 33.1–15 is shown, together with 50 bp of 5' and 3' flanking DNA (lower case). Differences from the consensus sequences are also shown for the individual numbered repeats (X, A or G; Y, C or T; –, missing nucleotide; >>>>, region not sequenced although clearly a tandem repeat of the consensus sequence, or of a close derivative of the consensus, by inspection of sequencing autoradiographs).

Methods. A library of 10–20-kb human DNA fragments cloned in bacteriophage λ L47.1 (refs 20,22) was screened by hybridization with 32 P-labelled pAV33.7 insert as described in Fig. 2. A random selection of eight positive plaques was purified to give recombinants λ 33.1–15. Each phage DNA was digested with *Hinf*I or *Hae*III, electrophoresed through a 1.5% agarose gel, and 33-repeat related sequences localized by Southern blot hybridization with pAV33.7 DNA (Fig. 2). Each recombinant gave a single positive *Hinf*I and *Hae*III fragment, except for λ 33.4 and 11 which gave no detectable positive *Hae*III fragments (because of the presence of a *Hae*III cleavage site in the repeat regions in these clones; data not shown). Suitable positive *Hinf*I and *Hae*III fragments were isolated by preparative gel electrophoresis³³, end-repaired if necessary and blunt-end ligated into the *Sma*I site of M13mp8 (ref. 37). M13 recombinants were isolated after transformation into *E. coli* JM101 and sequenced by the dideoxynucleotide chain-termination method^{38,39}. Each subcloned λ 33 fragment contained a tandem repetitive region which in some cases could be sequenced directly. In other cases where the repeat region was too far from the sequencing primer site, the M13 inserts were shortened by cleavage with suitable restriction endonucleases and resequenced.

estimated at 1.0×10^{-9} substitution per nucleotide site per year^{25–27}. Assuming that the generation time in man is 20 yr, this predicts a base substitution mutation rate of 2×10^{-5} per 1-kb minisatellite per gamete, lower than the estimated unequal exchange rate of 10^{-4} per gamete. This disparity in rates²³ is probably sufficient to maintain the amount of repeat sequence homogeneity seen in the hypervariable minisatellites λ 33.1, 4 and 6.

The rate of unequal exchange can therefore be as high as 10^{-4} per kb minisatellite sequence and presumably is proportional to minisatellite length. In contrast, the rate of homologous recombination at meiosis in human DNA is ~ 1 centimorgan per 10^6 bp (ref. 2) or 10^{-5} per kb. The apparently very high rate of unequal exchange in minisatellites suggests either that they are hotspots for meiotic recombination, or that most exchanges are between sister chromatids at mitosis in the germline.

A χ sequence in minisatellites?

The length and sequence of the consensus minisatellite repeat sequences vary considerably; none of them are flanked by direct repeats (Fig. 3), in contrast to the repeat region in the myoglobin gene (Fig. 1). Thus, it is unlikely that these minisatellites are related by transposition of a common ancestral sequence. We therefore used dot-matrix comparisons²⁸ of each minisatellite repeat consensus with the myoglobin 33-bp repeat sequence to determine which region(s) of the 33-bp repeat probe were detecting each minisatellite. Remarkably, the consensus sequence of

each minisatellite repeat aligns with the myoglobin repeat specifically over a unique 10–15-bp core region of the 33-bp probe sequence (Fig. 4). This shared core region consists of an almost invariant sequence GGCAGGAXG preceded by a 5-bp sequence common to most, but not all, repeats.

This core region in each cloned minisatellite suggests strongly that this sequence might help to generate minisatellites by promoting the initial tandem duplication of unique sequence DNA and/or by stimulating the subsequent unequal exchanges required to amplify the duplication into a minisatellite. As polymorphic minisatellites may also be recombination hotspots (see above), it might be significant that the core sequence is similar in length and in G content to the χ sequence, a signal for generalized recombination in *E. coli*²⁹ (Fig. 4a). Although the precise function of χ is unknown, current recombination models³⁰ suggest that this sequence binds the *recBC* gene product, endonuclease V, which unwinds locally and nicks DNA to produce a single-stranded DNA projection required for the generation of Holliday junctions. In principle, DNA repair synthesis from the nicking site, followed by ligation to the single-stranded DNA projection, could generate a short tandem duplication with each duplicate containing a χ sequence capable of promoting unequal exchange and amplification of the duplicated region to produce a minisatellite (Fig. 4b). Although this model is highly speculative, it predicts that isolated core (or core-like) sequences may also be hotspots for initiating human chromosome recombination.

Probe for hypervariable regions

The repeat length of each minisatellite region is usually half (λ 33.5 and 15), the same (λ 33.3, 6, 10 and 11) or double the length (λ 33.1 and 4) of the 33-bp probe from the human myoglobin gene (Fig. 3). This suggests that detection of minisatellites by pAV33.7 depends not only on the presence of a core sequence in each repeat but also on an in-phase alignment of cross-hybridizable core sequences in a heteroduplex between a minisatellite and the 33-bp repeat probe. If this is correct, then a probe consisting only of a tandem-repeated core sequence should be able to detect not only the human DNA fragments detectable by pAV33.7, but also additional minisatellites incapable of forming stable heteroduplexes with the 33-bp repeat probe. We therefore used the minisatellite from λ 33.15, comprising 29 almost identical repeats of an almost perfect 16-bp core sequence, as a hybridization probe for additional minisatellites.

As shown in Fig. 5, this repeated core probe detects a complex profile of hybridizing fragments in human DNA digested with *HinfI*, including most, but not all, of the bands detected previously with the 33-bp repeat probe from pAV33.7 (comparative data not shown). Only the largest (4–20 kb) *HinfI* fragments can be resolved fully; these show extreme polymorphism to the extent that the hybridization profile provides an individual-specific DNA 'fingerprint'. Large fragment hyperpolymorphism is to be expected as, if the rate of unequal exchange is proportional to minisatellite length, then long minisatellites (~10 kb long) will have a greater unequal exchange rate ($u \sim 0.001$), raising both the heterozygosity and the number of alleles in a population.

Pedigree analysis

To establish that these large highly polymorphic fragments are stably inherited and segregate in a mendelian fashion, we analysed *HinfI* digests of DNAs taken from an extensive Asian-Indian pedigree of Gujarati origin, including 54 individuals spanning four generations. The Southern blot/gel electrophoresis time was increased to improve the resolution of these large fragments. Typical examples of part of this pedigree are shown in Fig. 5d, e, confirming that the polymorphic variation is so great that all individuals, even in a single sibship of a first-cousin marriage (Fig. 5e), can be distinguished.

The families in Fig. 5d, e show that most of the large *HinfI* fragments are transmitted from each parent to only some of the offspring, establishing that most of these fragments are present in the heterozygous state and that the heterozygosity for these large hypervariable fragments must be approaching 100%. Furthermore, inheritance is Mendelian in that these heterozygous bands are transmitted on average to 50% of the offspring; 48 clearly-resolved different heterozygous parental bands were scored in four sibships of 4–6 individuals and gave a total of 116 cases in which an offspring had inherited a given parental band, compared with 124 cases where the band had not been transmitted (data not shown). Conversely, all fragments in offspring can be traced back to one or other parent, then in turn to their parents (with one exception, see below) and therefore provide a set of stably inherited genetic markers. No band is specifically transmitted from father to son or father to daughter (Fig. 5d), eliminating Y and X linkage respectively and implying that these minisatellite fragments are mainly autosomal in origin. Although it is not yet known from where in the set of autosomes these DNA fragments originate, they are not derived from a single localized region of one autosome. Instead, pairs of parental fragments can be identified which segregate independently in the offspring (Fig. 5d). Precisely, a pair of bands AB in one parent (and absent from the other) cannot be allelic, nor linked closely in repulsion, if there is at least one AB or — offspring; the presence of A— or —B recombinant progeny further establishes lack of tight linkage in coupling between A and B. Careful examination of the original autoradiograph of the family shown in Fig. 5d reveals, by these criteria, at least 10 resolvable bands in the mother, eight of which are mutually non-allelic and not

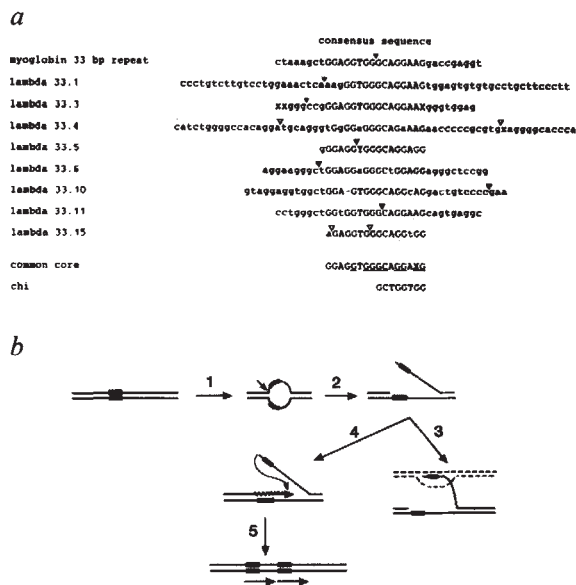


Fig. 4 A common χ -like core sequence shared by the repeat sequence of each minisatellite region. The sequence of each region was compared with the 33-bp tandem repetitive sequence in pAV33.7 and with its reverse complement, using dot-matrix analysis with variable windows and matching criteria²⁸. In each case, only a single unambiguous region of sequence similarity was found between the myoglobin 33-bp repeat sequence and the λ 33-repeat sequence. The same region was shared by the repeats of all eight λ 33 clones. **a**, The aligned sequence of each repeat consensus, each of which is given as an arbitrary cyclic permutation of the consensus shown in Fig. 3. The common core sequence shared by all repeats is also shown and positions in each consensus which conform to the core sequence are identified by upper case letters. Invariant nucleotides in the canonical core sequence are underlined; the generalized recombination signal (χ) of *E. coli* is given also²⁹. The beginning/end point of each repeat consensus (Fig. 3) is identified by ∇ ; in the case of λ 33.4 and λ 33.15, there is a non-integral number of repeats (Fig. 3) and the separate repeat beginning and end points are shown by ∇ . **b**, Model for minisatellite generation promoted by χ -like sequences. (1) A χ -like region denoted by a box. RecBC enzyme binds to χ and unwinds DNA. (2) Nicking produces a single-strand projection (3), which can be assimilated into a homologous duplex to form the precursor of a Holliday junction³⁰. (4) Alternatively, DNA repair synthesis followed by (5) ligation and segregation produces a tandem duplication of a χ -containing sequence which can amplify further by unequal exchange. The length of the tandem repeat is determined by the extent of repair synthesis.

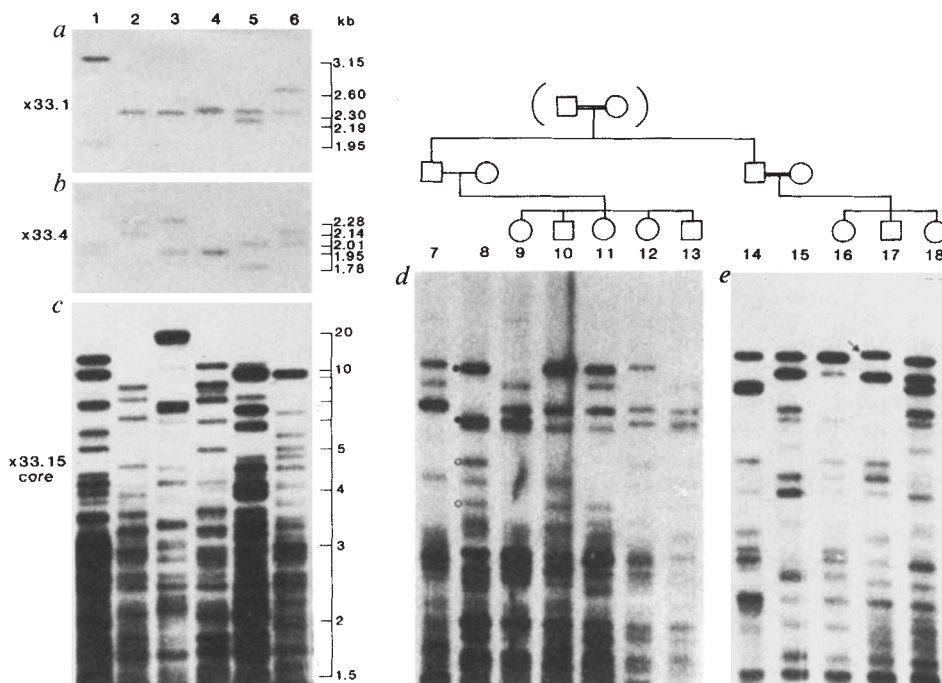
linked closely. Two other bands may each be an allele of one of the eight unlinked fragments, in that only A— and —B progeny are observed in the limited number of offspring analysed, although such a small sample is insufficient to prove that such pairs of fragments are alleles of a single locus. We conclude that the core probe can give useful information simultaneously on at least several distinct unlinked hypervariable loci.

A new mutant allele

The extreme variability of the large *HinfI* DNA fragments detected by the repeat core sequence suggests that the rate of generation of new length alleles must be very high and possibly amenable to direct measurement. There is a clear instance of a new mutation in the pedigree shown in Fig. 5e; individual 17 has a new fragment (arrowed), not present in either parent, which might have been derived by unequal exchange and slight expansion of a smaller maternal and paternal fragment present in the other offspring. In a survey of 27 individuals and their parents (data to be presented elsewhere), 240 clearly resolved offspring bands could be traced to one or other parent, the only exception being the band in individual 17. This gives a mutation rate u to a new allele for these hypervariable fragments of

Fig. 5 Polymorphic human DNA fragments detected by hybridization with individual λ 33 probes. Southern blots of *HinfI* digests of DNA from a random sample of British caucasians (1–6) and from selected members of a large British Asian-Indian pedigree (7–18) were hybridized with single-stranded 32 P-labelled hybridization probes prepared from suitable M13 recombinants containing minisatellite regions. The pair of bands (○) in individual 8 is an example of non-allelic fragments which are not tightly linked; the pair marked (●) illustrates possible allelism in that each of the five offspring inherits only one of the two fragments. The arrowed fragment in individual 17 is present in neither parent and is a new mutant. The correct paternity of individual 17 has been verified as described below.

Methods. 10 μ g samples of DNA prepared from white blood cells¹⁰ were digested with *HinfI*, electrophoresed through a 20-cm long 1% agarose gel and transferred by blotting to a Sartorius nitrocellulose filter. Single-stranded 32 P-labelled hybridization probes were prepared from M13 recombinants as follows. Approximately 0.4 μ g M13 single-stranded DNA was annealed with 4 ng 17-mer sequencing primer⁴⁰ in 10 μ l 10 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.0) at 60 °C for 30 min. Primer extension was performed by adding 16 μ l 80 μ M dATP, 80 μ M dGTP 80 μ M TTP, 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA plus 3 μ l (30 μ Ci)[α - 32 P]dCTP (3,000 Ci mmol⁻¹) and 1 μ l 5 units μ l⁻¹ Klenow fragment (Boehringer) and incubating at 37 °C for 15 min. Extension was completed by adding 2.5 μ l 0.5 mM dCTP and chasing at 37 °C for a further 15 min. The DNA was cleaved at a suitable restriction endonuclease site either in the insert or in the M13 polylinker distal to the insert, denatured by adding 1/10 vol. 1.5 M NaOH, 0.1 M EDTA, and the 32 P-labelled single-stranded DNA fragment extending from the primer was recovered by electrophoresis through a 1.5% low melting point agarose gel (Sea Plaque). The excised band (specific activity > 10⁹ c.p.m. μ g⁻¹ DNA) was melted at 100 °C in the presence of 1 mg alkali-sheared carrier human placental DNA (sheared in 0.3 M NaOH, 20 mM EDTA at 100 °C for 5 min) and added directly to a pre-warmed hybridization chamber; the carrier DNA also suppressed any subsequent hybridization to repetitive DNA sequences. The precise probes used were: 33.1, a 2,000-nucleotide subcloned *HaeIII* fragment containing the minisatellite plus 350-nucleotide flanking human DNA; 33.4, a 695-nucleotide non-minisatellite *EcoRI* fragment on the primer-proximal side of the minisatellite contained in a 2,015-nucleotide *HinfI* fragment; 33.15 core, a 592-nucleotide subcloned fragment containing the minisatellite sequence plus 128-nucleotide flanking human DNA. Hybridizations were performed as described elsewhere³⁶, except that dextran sulphate was replaced by 6% (w/v) polyethylene glycol 6,000 (Fisons) to reduce background labelling⁴¹. Filters A and B were hybridized overnight in 0.5 $\times$ SSC at 65 °C and washed in 0.2 $\times$ SSC at 65 °C. Filters C–E were hybridized and washed in 1 $\times$ SSC at 65 °C. Filters were autoradiographed for 1–3 days at –80 °C using a fast tungstate intensifying screen. The correct paternity of individual 17 was established using a range of biochemical and blood group markers (haptoglobin, transferrin, red cell acid phosphatase, phosphoglucomutase I, adenylate kinase, adenosine deaminase, α_1 -antitrypsin, G_c, G_m, esterase D, glyoxylase, phosphoglycolate phosphatase, C3, peptidase D, ABO, Rh and HLA; S.L.T., unpublished data), and confirmed further by rehybridizing this blot with the core minisatellite in λ 33.6 to generate a second DNA 'fingerprint' in which all polymorphic bands in individual 17 could be traced back to one or other parent (data not shown).



$\sim \frac{1}{240} = 0.004$. This estimate is in reasonable agreement with the population genetic estimate of $u \sim 0.001$ for these very large hypervariable fragments (see above).

Conclusions

Here, we show not only that it is possible to design probes for the cloning of individual polymorphic minisatellite regions from human DNA, but also that the shared core sequence, which possibly serves as a recombination signal and promotes the formation of minisatellites, can be used for the simultaneous analysis of multiple hypervariable regions. We anticipate that these DNA 'fingerprints' will be of general use in human segregation analysis, in particular for detecting specific bands in close linkage with disease loci in large pedigrees and for studying marker loss in tumours. In addition, they provide a powerful method for paternity and maternity testing, can be used in forensic applications and might also be useful in detecting inbreeding between couples who have had an affected offspring possibly caused by an autosomal recessive gene carried by both parents.

The precise sequence of the core consensus shared by the repeat elements of the cloned minisatellites will be biased by the particular version of the core present in the myoglobin gene minisatellite used as the initial hybridization probe. Therefore, other variant (core)_n probes might detect additional polymor-

phic loci not found by the λ 33.15 repeated core sequence. Preliminary experiments have indeed shown that the core minisatellites in λ 33.5 and λ 33.6 also hybridize to multiple hypervariable loci, many of which are novel. We are attempting currently to clone large hypervariable regions to provide locus-specific probes for individual minisatellites.

The detection of new mutant-length alleles of minisatellites in human pedigrees will help the analysis of rates and mechanisms of unequal exchange and gene homogenization and can in principle be used to determine whether such exchanges occur by sister chromatid exchange or by recombination between homologous chromosomes at meiosis. In addition, if the core sequence is indeed a recombination signal, then its accurate definition could provide useful substrates for studying mechanisms of human recombination.

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LETTERS TO NATURE

Cometary impacts, molecular clouds, and the motion of the Sun perpendicular to the galactic plane

Patrick Thaddeus*† & Gary A. Chanan†

* Goddard Institute for Space Studies, 2880 Broadway, New York, New York 10025, USA

† Columbia Astrophysics Laboratory, Department of Physics, Columbia University, New York, New York 10027, USA

Raup and Sepkoski¹ have presented evidence from marine fossils for a 26-Myr periodicity in the occurrence of mass extinctions. Using the same data Rampino and Stothers² obtained a different period, 30 ± 1 Myr, which agrees with the 33 ± 3 -Myr half-period for the vertical oscillation of the Solar System about the plane of the galaxy³. To explain this agreement they suggest² that encounters with molecular clouds perturb the Sun's family of comets, causing many to enter the inner Solar System where one or more collide with the Earth; the cloud encounter rate is modulated at twice the oscillation frequency, because the number density of clouds peaks at the galactic plane at the midpoint of the solar oscillation crossed by the Solar System twice per period. Notwithstanding an apparent objection to this that the most recent extinctions are not in phase with the solar oscillation⁴, their model, given its stochastic nature, can accommodate a few events with large phase discrepancies. The degree of modulation is crucial: it depends on the scale height of the population of molecular clouds relative to the amplitude of the solar motion and tends to zero if this ratio is large and encounters are entirely random. Here we present data from CO surveys of molecular clouds both within and beyond the solar circle, which permit explicit calculation of the strength of the modulation. The cloud layer near the Sun is too extended and, as a consequence, the modulation of cloud encounters is too weak for a statistically significant period to be extracted from the nine extinctions analysed by Rampino and Stothers.

Although we assume a gaussian distribution of clouds with z , the distance from the galactic plane, our conclusions do not depend sensitively on this assumption and remain essentially unchanged if an exponential or other plausible distribution function is assumed instead. The empirical parameter crucial to the mechanism of Rampino and Stothers is, then, the ratio of the amplitude of the solar oscillation z_0 to the half-thickness at half-density $z_{1/2}$ of the distribution of local molecular clouds. One is not free to treat z_0 as a free parameter; it is fixed by (1)

the requirement that the period, T , of the oscillation must be 60 Myr, twice the putative extinction period, (2) the circumstance that the Sun is now close to the galactic plane so its z component of velocity, $v_z = 7.4 \text{ km s}^{-1}$, is essentially that at $z = 0$, and (3) the constraint of simple harmonic oscillation⁵, $z_0 = v_z T / 2\pi = 72 \text{ pc}$.

Because of the large scatter and uncertainty in the distance of local clouds, there is, as yet, no reliable measurement of $z_{1/2}$ in the vicinity of the sun (that is, within $\sim 1 \text{ kpc}$) from CO or other molecular cloud surveys, but there are good measurements from several large-scale CO surveys of $z_{1/2}$ as a function of galactocentric distance for distant clouds both within the solar circle and beyond. Figure 1 summarizes the survey data. All the CO surveys are consistent with a gradual increase of $z_{1/2}$ with R , proportional approximately to $R^{0.5}$ and a value of $z_{1/2}$ at the solar circle of $85 \pm 20 \text{ pc}$ (uncertainty 1 s.d.) which we will adopt here for the solar vicinity. Local observations of clouds at known distances are consistent with this value, but are inconsistent with a population of clouds significantly more compressed to the galactic plane. The well-known large concentrations of molecular clouds in Orion and Monoceros⁶, for example, representing a significant fraction of local clouds by mass, lie 150–200 pc from the plane, or about twice our adopted $z_{1/2}$.

To calculate the encounter rate as the solar system oscillates sinusoidally through a gaussian distribution of clouds, we may safely neglect the small present displacement of the Sun from the galactic plane. The solar velocity components parallel and perpendicular to the plane are then $v_{\parallel} = v \cos b = 18.5 \text{ km s}^{-1}$ and $v_{\perp} = v \sin b \sin \omega t = 7.4 \text{ km s}^{-1} \sin \omega t$, where $v = 20 \text{ km s}^{-1}$ is the present solar motion relative to local stars and interstellar matter⁷, $b = 22^\circ$, the galactic latitude of the solar apex⁷, and $\omega_0 = 2\pi/60 \text{ Myr}^{-1}$. Because of the low latitude of the apex, the solar speed as a function of time, $v(t) = v_{\parallel}(1 + 0.164 \cos^2 \omega_0 t)^{1/2} \approx v_{\parallel}(1 + 0.082 \cos^2 \omega_0 t)$, is always large with respect to the random motion of the clouds, which may be neglected. Letting $\zeta = \ln 2 z_0^2 / z_{1/2}^2$, the number of encounters (extinctions) per unit time is then simply

$$r(t) = n(z(t))v(t)\sigma \\ = C(1 + 0.082 \cos^2 \omega_0 t) \exp(-\zeta \sin^2 \omega_0 t) \quad (1)$$

an explicit function of time at a given ζ with no free parameters except the constant of normalization $C \equiv n_0 v_{\parallel} \sigma$, where n_0 is the in-plane number density of clouds and σ the encounter cross section. It is tacitly assumed that the encounters are short range, that is, that the impact parameter b is small with respect to $z_{1/2}$; the effect of long range encounters is clearly to reduce the modulation (see below).

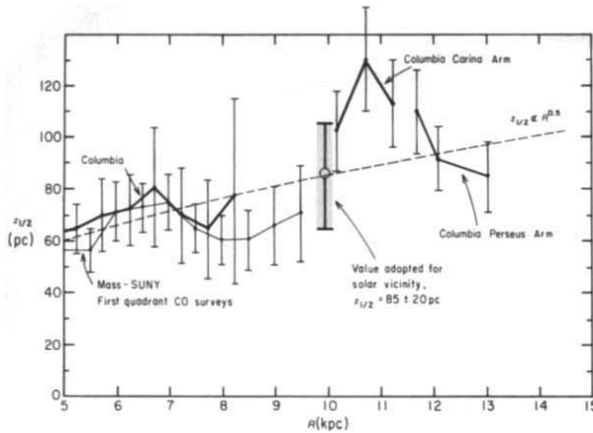


Fig. 1 Half-thickness of the distribution of molecular clouds with distance from the Galactic plane, as a function of distance from the centre of the Galaxy, determined by the Massachusetts-Stony Brook (ref. 11) and Columbia CO surveys.

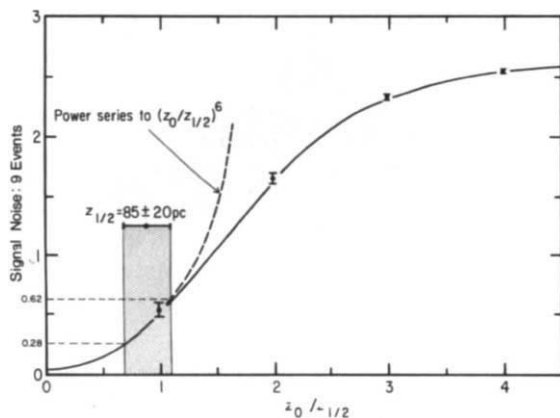


Fig. 2 Signal-to-noise of the leading Fourier component at $2\omega_0$ for nine events, as a function of the ratio of the amplitude of the solar oscillation to the half-thickness of the molecular cloud disk. The four points with vertical error bars indicate the results of a check on equation (4) with the Monte-Carlo code used to produce Fig. 3.

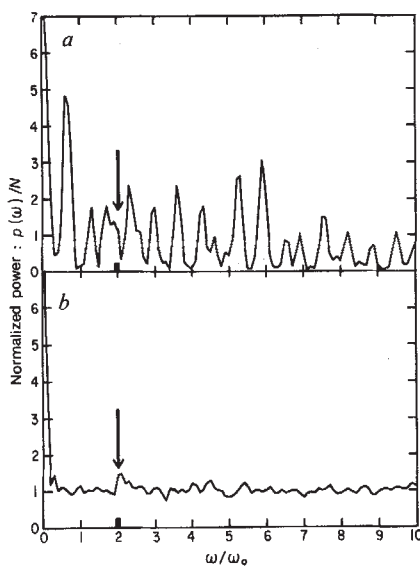


Fig. 3 Monte-Carlo calculation for $z_0/z_{1/2} = 1$ of the power spectrum of random events drawn from the distribution given by equation (1). *a*, Nine events, the number of mass extinctions analysed by Rampino and Stothers²; *b*, the average of 100 such spectra, showing the small expectation value $\langle p(R) \rangle - \langle p(0) \rangle$ of the nine-event peak (short vertical bar at $\omega/\omega_0 = 2$).

We now wish to calculate the power spectrum $p(\omega)$ for a sample of N encounters drawn from this distribution with respect to time, and, specifically, the signal strength of the leading Fourier component at $2\omega_0$ relative to the shot noise, that is, the purely statistical fluctuations in the absence of modulation. We define the complex Fourier amplitude as

$$A(\omega) = \sum_{i=1}^N \exp(j\omega t_i) \equiv \sum_{i=1}^N (x_i + jy_i) \quad (2)$$

where the t_i are the encounter times and define the power spectrum as usual as $p(\omega) = |A(\omega)|^2$. The first and second moments of $A(2\omega_0)$ are readily calculated. We may take $\langle y \rangle = 0$ without loss of generality. Then, $\langle x \rangle = \int r(t) \cos 2\omega_0 t dt / \int r(t) dt \equiv R$, $\langle x^2 \rangle = \int r(t) \cos^2 2\omega_0 t dt / \int r(t) dt \equiv S^2$ and $\langle y^2 \rangle = 1 - S^2$, and the variances corresponding to the second moments are $\sigma_x^2 = S^2 - R^2$ and $\sigma_y^2 = 1 - S^2$. The expectation value of the power at $2\omega_0$ is then

$$\langle p(R) \rangle = N^2 \langle x \rangle^2 + N \sigma_x^2 + N^2 \langle y \rangle^2 + N \sigma_y^2 = N(N-1)R^2 + N \quad (3)$$

Care is required in calculating the signal-to-noise ratio (S/N) as the statistical fluctuations in the absence of modulation (that is, signal) are distributed normally in A but exponentially in p ; the expression for this ratio in the absence of knowledge of the phase of the signal is

$$S/N = \left[\frac{\langle p(R) \rangle - \langle p(0) \rangle}{\langle p(0) \rangle} \right]^{1/2} = \sqrt{N-1} R \quad (4)$$

The normalized Fourier transform R in equation (2) may be conveniently approximated analytically when ζ is not too large, by expanding the exponential in equation (1) in a power series, or it may be readily evaluated numerically at all ζ . The S/N ratio from both methods for $N=9$ events is shown in Fig. 2. For the observed thickness of the population of molecular clouds at the solar circle, S/N is evidently so small, 0.45 ± 0.17 , that a statistically significant determination of the underlying modulation period cannot be obtained.

A graphic demonstration of this conclusion and a useful check as well of the analytical theory (Fig. 2), has been obtained by Monte Carlo simulation. The top of Fig. 3 shows the power spectrum derived numerically from a sample of nine random events drawn from the distribution of equation (1); the signal is clearly swamped by the purely statistical fluctuations, that is, the shot noise spikes. The expectation value of the peak power can be obtained by averaging together 100 such (independent) spectra, as in the bottom panel of Fig. 3; its smallness compared with the noise spikes in the top panel is evident. To express this result another way, scaling from equation (4) we find that even a relatively modest signal characterized by $S/N=3$ would require not nine extinction events, but > 300 .

Given that our assumption, that the encounters between the Solar System and molecular clouds are short range with respect to $z_{1/2}$, is an oversimplification, a significantly larger number of events may actually be required. Because of the fairly flat mass spectrum of molecular clouds^{8,9}, the most important encounters are likely to be with large clouds or cloud complexes whose mass exceeds $1 \times 10^5 M_\odot$ and these will occur mainly at impact parameters exceeding 50 pc (ref. 10). The result of such distant encounters is to increase significantly the effective thickness of the molecular cloud layer, which, by Fig. 2, further reduces the S/N ratio in the power spectrum of the peak at $2\omega_0$. Because of the breakdown of the impulsive approximation at large impact parameters, exactly how many additional events are required when this effect is taken into account is not clear, but we estimate that it could be a factor ≥ 10 . Our conclusion is, therefore, that at least 300 mass extinctions and possibly many more, as opposed to the nine analysed by Rampino and Stothers, are required to yield statistically significant evidence for the oscillation of the Solar System about the plane of the galaxy under the molecular cloud hypothesis. It follows that if a periodicity does exist in the data, it is highly unlikely to result from encounters between the Solar System and molecular clouds.

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A composite bolometer as a charged-particle spectrometer

N. Coron*, G. Dambier*, G. J. Focker†, P. G. Hansen‡, G. Jegoudez*, B. Jonson§, J. Leblanc*, J. P. Moalic*, H. L. Ravnt†, H. H. Stroke|| & O. Testard¶

* LPSP, BP 10, F-91371 Verrières-le-Buisson, France

† EP-Division, CH-CERN, 1211 Genève 23, Switzerland

‡ Institute of Physics, University of Aarhus,

DK-8000 Aarhus, Denmark

§ Chalmers University of Technology, S-41296 Göteborg, Sweden

|| New York University, Department of Physics, New York, New York 10003, USA

¶ Service d'Astrophysique, CEN, Saclay, F-91111 Gif-sur-Yvette, France

The measurement of radioactivity by direct conversion of nuclear radiation into a temperature rise of a calorimeter is as old as nuclear physics itself. As part of a general programme aiming at a determination of the mass of the electron neutrino, we have designed an improved version of a He-cooled composite diamond bolometer with a monolithic germanium thermistor, developed at the Laboratoire de Physique Stellaire et Planétaire (LPSP)¹. Our approach, based on an idea by De Rújula², is to study the shape, near the upper end-point of the internal bremsstrahlung spectrum in electron-capture β decay. The best nucleus for a precise measurement seems to be ¹⁶³Ho, for which we have determined³ the Q_{EC} value to be 2.83 ± 0.05 keV. A particularly interesting possibility is to use total absorption spectrometry⁴ (calorimetry), in which the radioactive holmium forms part of the sensitive volume of the detector. With 5–6-MeV α particles impinging on the diamond wafer of the bolometer, a full-width-at-half-maximum (FWHM) of 36 keV was obtained at a temperature of 1.3 K. The theoretical resolution at 100 mK is a few electron-volts, so this new detection technique should give greatly enhanced energy resolution compared with present solid-state conductors based on charge carrier collection.

In 1903 Curie and Laborde⁵ used a calorimeter to verify that the heat produced by a radioactive substance results from the absorption of its energetic radiation. After this, microcalorimetry became of great theoretical importance through the determination⁶ of the 0.337-MeV average β energy of ²¹⁰Bi (Ra E). In fact, the contrast between this value and the maximum β -decay energy of 1.17 MeV for ²¹⁰Bi was one of the key arguments which led Pauli to the hypothesis of the neutrino. Microcalorimetry finally reached a sensitivity of $\sim 3 \times 10^{-5}$ W at 300 K⁷. It was Simon⁸ who first pointed to the possibility of nuclear micro-calorimetry at low temperatures, where heat capacities are very low; Dalmazzone⁹ has investigated a calorimeter at 1.8 K and reached a sensitivity of 10^{-9} W. The idea that bolometers or thermometers at low temperature could be used as detectors for radioactivity has reappeared

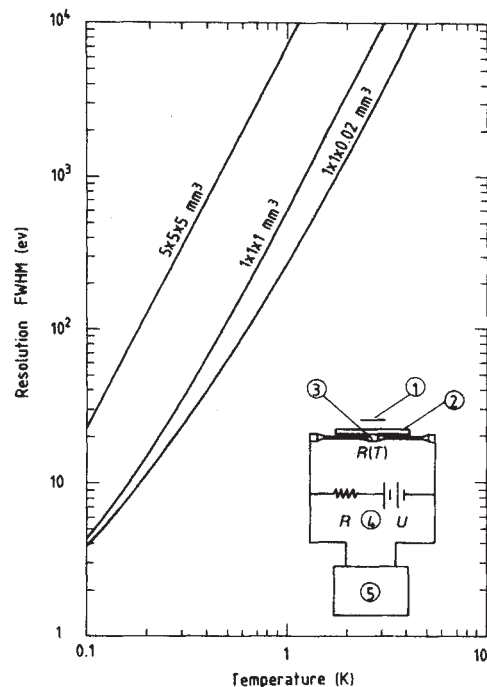


Fig. 1 Ultimate resolution with optimal filtering as a function of the temperature for the best available composite bolometer with a monolithic thermistor. The curves are calculated for three different detector volumes, V (mm^3), by means of the expression for Δu , given in the text. The 0.02-mm-thick detector represents the present technological size limit. The specific heat, C , is strongly material- and geometry-dependent and we have used the expression, $C = 7 \times 10^{-12} T^3 + 1.3 \times 10^{-12} T^{1.3} + 1.5 \times 10^{-12} T + 6.8 \times 10^{-11} VT^3$ as a good approximation to available measurements. The inset shows a schematic view of the experiment: (1) the α source; (2) the diamond substrate; (3) the Ga-doped Ge-thermistor, which also serves as mechanical support and thermal conductor to the 1.3 K thermostat; (4) the bias supply and the cooled load resistor; (5) the preamplifier-amplifier chain (at room temperature).

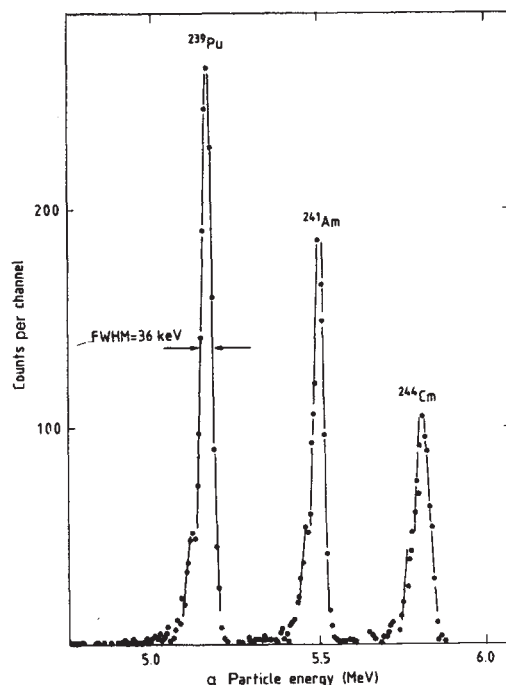


Fig. 2 Alpha energy spectrum from a mixed source of ²³⁹Pu, ²⁴¹Am and ²⁴⁴Cm, obtained by recording the thermal pulses induced by the α particles in a 0.25-mm³ diamond bolometer. The resolution is 36 keV (FWHM) and the integral non-linearity is $< 10^{-3}$.

recently¹⁰⁻¹² and it has been shown that a silicon thermistor may be used as an X-ray spectrometer¹¹.

The traditional use of low-temperature bolometers is for the detection of a continuous flux of infrared radiation¹³⁻¹⁸. The instruments based on semiconductors were found to have better performance and to be simpler to use than those based on superconductivity. The semiconductors (doped Ge or Si) have a resistance varying as T^{-4} – T^{-9} in the temperature region of 0.3–5 K. In the early 1970s a new type of bolometer, a so-called composite one, was developed at LPSP^{1,14}, consisting of an absorber with a large surface area (0.5–20-mm diameter) coupled thermally to a monolithic semiconductor thermistor with very small volume (typically, 0.008 mm³). Its best performance has been obtained with a diamond wafer as the absorber and a germanium crystal as thermometer. (The use of diamond is especially advantageous because of its very high thermal diffusivity¹.) A schematic diagram of a composite bolometer is shown as an inset in Fig. 1. An asset of this construction is that there is no need for soldering, which results in a minimum of low frequency noise and a low heat capacity.

The essential advantage of the composite bolometer is that it separates the absorbing and detecting functions. The absorber (diamond, sapphire, dielectric-metal sandwich) may be optimized independently of the thermometer, which in turn has to have maximal temperature derivative of the impedance, dZ/dT , and the impedance, Z , matched to the preamplifiers¹⁸. The use of bolometers for detection of different kinds of flux is increasing steadily: for molecular beams^{19,20}, for radiation in the millimetre wave-length range¹⁴⁻¹⁸ and, most recently, for X rays¹¹. It is also interesting to note that spurious pulses, caused by cosmic-ray absorption in the infrared low-temperature detectors, were deliberately eliminated, using a 'spike suppression circuit', by high-altitude astronomers²¹.

The diamond wafer, which acts as the absorber in our bolometer, has a volume 0.25 mm³. At our working temperature (1.3 K) we have measured the thermalization time of the device to be <60 μ s from any point of impact on its surface. The thermometer, which has a surface area of 0.1×0.1 mm², is glued to the diamond with a 10- μ m layer of epoxy, which eliminates the possibility of charge collection, as there is no bias across the absorber.

The resolution of the detector is only limited by the noise of the detector itself (Johnson noise and thermodynamic noise)(see refs 10 and 18 for a detailed analysis of these noise effects). With optimal filtering, an order of magnitude estimate for the resolution is given by $\Delta U_{FWHM} = \alpha \sqrt{8 (\ln 2) k_B T^2 C}$ where k_B is the Boltzmann constant, T the temperature of the helium bath (in K) and C the specific heat of the bolometer at the temperature T . The constant α depends on the details of the construction of the bolometer (on dZ/dT and Z) and generally has a value of 1–2; here, $\alpha = 1.5 \pm 0.2$. The limiting resolution calculated from this expression is shown in Fig. 1.

The bolometer used in this experiment is mounted in the vacuum chamber of a commercial portable optical cryostat²². A mixed ²³⁹Pu, ²⁴¹Am, ²⁴⁴Cm α source was positioned in front of the diamond so that no particles could reach the Ge thermometer. A Pb shutter could be inserted between source and detector during background measurements. A typical α spectrum measured at a temperature of 1.3 K is shown in Fig. 2. The low-noise preamplifier had a band pass filter of 2–120 kHz and a gain of 2,000 (ref. 23). A 5.5-MeV α particle gives a typical temperature increase of 1 mK. The resolution is 36 keV, which is not yet the best possible performance of the system. The main reason for this is that we have used standard nuclear electronics which could not be optimally matched to the time constant of the bolometer. We are presently working on improvements to both the electronics and the cryostat and hope soon to have an operational α detector with a resolution of ~ 3 keV at 1 K. We foresee that the technique of nuclear radiation detection via thermal pulses will have a great impact on many fields of physics.

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An apparently first-order transition between two amorphous phases of ice induced by pressure

O. Mishima, L. D. Calvert & E. Whalley

Division of Chemistry,
National Research Council,
Ottawa, Canada K1A 0R6

We recently reported¹ a transition from ice Ih to a high-density amorphous phase at 10 kbar, 77 K. Here we report that low-density amorphous ice (density 0.94 g cm⁻³) compressed at 77 K transforms to high-density amorphous ice (1.19 g cm⁻³ at zero pressure) at a sharp transition at 6 ± 0.5 kbar. The transition is at least as sharp as the previously reported¹ transition and strongly resembles a first-order transition in its sharpness and large volume change. It appears to be the first example of an apparently first-order transition between amorphous solids and has implications not only for our understanding of the behaviour of condensed matter, but also for theories of planetary interiors.

Ice Ih at 77 K, when compressed to its extrapolated melting pressure of 10 kbar, transforms very readily (in view of the temperature) to an amorphous solid of density 1.31 ± 0.02 g cm⁻³ at 10 kbar and 1.17 ± 0.02 g cm⁻³ at zero pressure¹. The transformation seems to be of a new kind, in which a solid 'melts', at an apparently first-order transition well within the glass region of the liquid, to produce an amorphous phase that resembles the glass at the temperature and pressure of the experiment. When heated at zero pressure, the new phase transforms at ~ 117 K to an amorphous phase whose X-ray diffraction pattern resembles that of the amorphous ice made by condensing the vapour on a cold surface^{2,3}. We have since determined the density of this phase, by weighing in liquid nitrogen, to be 0.94 ± 0.02 g cm⁻³.

The low-density amorphous ice would no doubt become less stable than the high-density amorphous ice it was made from, if it were put under high enough pressure. Eventually, it might transform to a high-density amorphous form resembling that made by squeezing ice I, perhaps at an apparently first-order transition resembling that of ice I' and, if so, its density would increase by $\sim 23\%$. Such an apparently first-order transformation of one amorphous phase to another with a large increase in density seems not to have been reported, although gradual

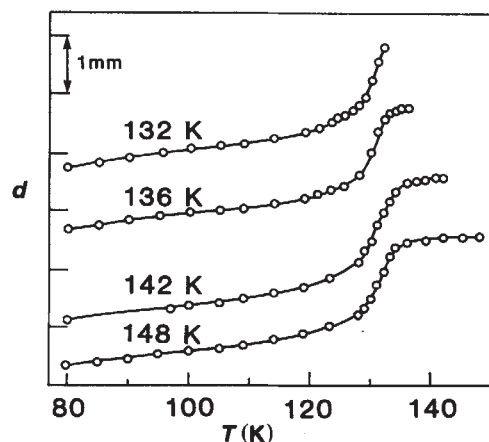


Fig. 1 The evolution of low-density amorphous ice from the high-density amorphous. High-density amorphous ice was prepared by compressing about 1.0–1.2 cm³ of water in an indium cup at 77 K to ~16 kbar in a steel vessel of 12.5 mm inside diameter, 50-mm outside diameter and 75-mm length, using a hydraulic press¹, and the pressure was reduced to a nominal pressure of 100 bar. The samples were heated in the pressure vessel at the rate of ~1 K min⁻¹ to 132, 136, 142 and 148 K, as marked on the curves, and then quenched by immersing in liquid nitrogen. The temperature was measured by a thermocouple attached to the pressurizing piston and the sample may be slightly cooler than the thermocouple. The displacement of the piston is plotted against temperature in the figure.

densifications of amorphous phases of up to 20%, when squeezed to ~100 kbar with or without shearing, have been reported⁴⁻⁹. The compression of amorphous ice, reported here, should therefore be very interesting.

Four samples of high-density amorphous ice were made by compressing ice I at 77 K in an indium cup inside a steel pressure vessel¹ to ~16 kbar. The pressure was then reduced to 0.1 kbar and the pressure vessel and its contents were heated to 132, 136, 142 or 148 K at a rate of ~1 K min⁻¹ to transform the high-density amorphous ice into the low-density amorphous; the increase in volume was followed by the displacement of the piston. The sample was then cooled rapidly to 77 K.

The piston displacement during each of the four runs is plotted against the temperature in Fig. 1. The displacement changes rapidly with temperature above ~128 K because the high-density amorphous ice is transforming to low-density. The samples heated to 136, 142 and 148 K, which contained significantly different amounts of water, seem to have been completely transformed to the low-density form in the time of the experiment, but the sample heated to 132 K was incompletely transformed.

The low-density amorphous ice was then compressed and decompressed at 77 K in the same apparatus. The displacement of the piston during the compressions and decompressions is plotted in Fig. 2, where it is compared with the compression of a similar amount of ice Ih¹ (lowest curve). All the low-density amorphous samples compressed elastically until the sample pressure, corrected for friction, reached 6 ± 0.5 kbar (the uncertainty is mainly due to friction in the apparatus), when a rather sudden (in view of the low temperature) transition started and the volume decreased by 4.0 ± 0.3 cm³ mol⁻¹ or ~22%. Half of the compression occurred in a pressure range of ~0.5 kbar and the transformation seemed complete at 8–9 kbar. The transformation did not reverse when the pressure was decreased and the sample expanded only elastically. The samples that had been prepared by heating to 142 and 148 K transformed at the 6-kbar transition, but underwent another inelastic compression starting at ~10 kbar. No doubt some ice Ih¹ or Ic was formed when the samples were heated; when the samples were compressed, the ice transformed to high-density amorphous ice at 10 kbar¹, as is the case for ice Ih.

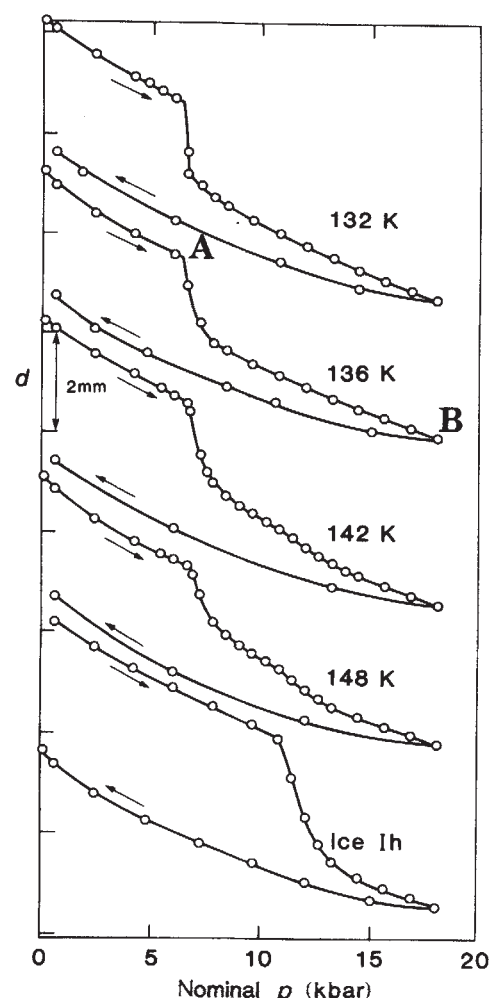


Fig. 2 Compression at 77 K to 16 kbar and decompression of the four samples of low-density amorphous ice. They were made as described in Fig. 1 legend. The arrows show the direction of the pressure changes, and the samples are identified by the temperature at which they were formed. The compression of ice Ih at 77 K is plotted as the lowest curve for comparison.

The density of the high-density phase at 6 kbar was estimated from the piston displacement to be 1.26 ± 0.02 g cm⁻³, and 1.19 ± 0.02 g cm⁻³ at zero pressure. The zero-pressure density is equal, within the experimental uncertainty, to that (1.17 g cm⁻³) of the amorphous made by transforming ice Ih at 77 K and zero pressure.

Samples were recovered from the conditions represented by points A and B of the 136-K run in Fig. 2 and microphotometer traces of their X-ray diffraction patterns are reproduced in Fig. 3. The pattern of sample A is, within the experimental uncertainty, the same as that of low-density amorphous ice¹ and the pattern of sample B is the same as that of the high-density amorphous ice made by compressing ice Ih at 77 K to 10 kbar¹. There is no doubt that the phase produced by the 6-kbar transition of low-density amorphous ice at 77 K is another amorphous phase and is similar to the high-density amorphous ice made by transforming ice I at 10 kbar¹.

The seemingly almost discontinuous transition from low-density to high-density amorphous ice with a decrease of volume of ~22% resembles, in its sharpness, the 'melting' of ice I at 77 K and 10 kbar to the 'liquid' in the glass region¹. It seems that the low-density amorphous ice becomes unstable, relative to the high-density amorphous phase, perhaps at its surface, where ice I seems to become unstable¹, and undergoes a new kind of apparently discontinuous transition between two amorphous phases which at least approximates first-order behaviour. The phase that is produced strongly resembles the amorphous

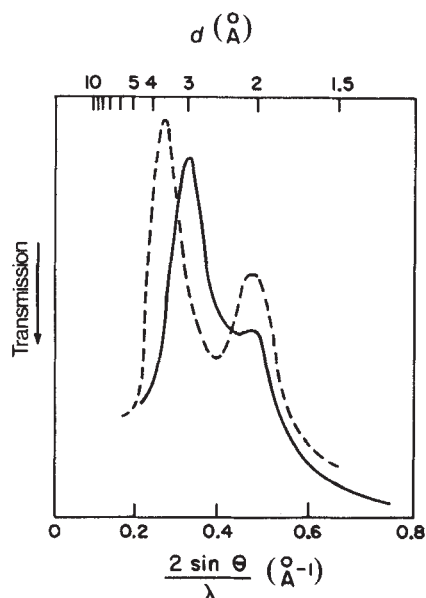


Fig. 3 Representative microphotometer tracings as a function of $(2 \sin \theta)/\lambda$, where 2θ is the scattering angle and λ , the wavelength of the X rays, of diffraction patterns of homogeneous samples of low-density amorphous ice (dashed line) and of the high-density amorphous ice (solid line) taken in a flat-plate X-ray camera. The temperature was controlled to ± 5 K by flowing nitrogen gas and was measured by a thermocouple placed near the specimen. Photographs were taken at ~ 95 K using zirconium-filtered molybdenum radiation.

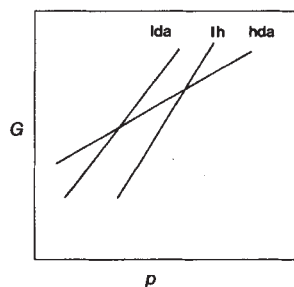


Fig. 4 Schematic graph of the Gibbs function G against the pressure p for ice Ih and low-density (lda) and high-density (hda) amorphous ice.

ice made by transforming ice I, and the transition seems to be a new way of making amorphous phases.

The pressure of the amorph-amorph transition is 4 kbar lower than that of the transition of ice I to amorphous ice at the same temperature. The difference in the transition pressures is readily understood if both transitions are considered as first-order, as they seem to be to a good approximation because they are so sharp and occur with a large volume change. The specific Gibbs functions of ice Ih, low-density amorphous ice and high-density amorphous ice are plotted schematically against the pressure in Fig. 4. From the equation $dG/dp = V$, it is readily shown that the Gibbs-function difference for two phases at zero pressure which are in equilibrium at pressure p_e is, to first order and neglecting the compressibility difference of the two phases, $\Delta G(p=0) = -\Delta V(p=p_e)$, where ΔV is the volume difference of the two phases. The value of p_e for transformation to the high-density amorphous phase will be tentatively assumed to be the experimental transformation pressure. From the transformation pressure of ice Ih to high-density amorph¹ (hda) and of low-density (lda) to high-density amorph, and from the specific volumes of the phases at zero pressure, the Gibbs-function differences at zero pressure are $\Delta_{lh}^{hda} G(p=0) = 4.1 \pm 0.3$ and $\Delta_{lda}^{hda} G(p=0) = 2.5 \pm 0.3$ kJ mol⁻¹, so that $\Delta_{lh}^{lda} G(p=0) = 1.6 \pm 0.3$ kJ mol⁻¹.

A similar transformation ought to occur in other four-coordinated amorphous solids, such as amorphous silicon and germanium. These materials crystallize when compressed at room temperature^{10,11}, but may form high-density amorphous phases if compressed at low temperature.

The ultimate phase of ice, as far as is known, is ice VII or VIII, both based on a body-centred-cubic arrangement of the oxygen atoms, and the corresponding phase having centrosymmetric hydrogen bonds, known as ice X^{12,13}. The density of ice VIII when recovered at zero pressure and 77 K is 1.46 g cm⁻³ (ref. 14), about 25% denser than our high-density amorphous ice. The high-density amorphous ice may, therefore, undergo yet another transformation, either gradually or suddenly, if it is subjected to high enough pressures. We are presently testing this suggestion.

Finally, this amorphous-amorphous transition may be important in the planets. The ice formed in space from gaseous molecules is very probably amorphous¹⁵ and if it agglomerates to form a planet, it will transform to the high-density amorph if the planet becomes large enough, that is, having a radius of the magnitude of 2 Mm.

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Al³⁺ coordination changes in liquid aluminosilicates under pressure

Eiji Ohtani*†, Francis Taulelle‡ & C. Austen Angell§

* Research School of Earth Sciences, Australian National University, Canberra, ACT, Australia

‡ Laboratoire de Physique Thermique, ESPCI, 10 Rue Vauquelin, 75005 Paris, France

Geochemists often debate the conditions in which Al³⁺ may become 6-coordinated in molten silicates and how such liquid structures are related to those of coexisting crystal phases¹⁻⁴. To date no experimental evidence for the occurrence of Al³⁺ in this coordination state has been presented and computer simulation studies have suggested that pressures exceeding 100 kbar may be required for the 4→6 conversion^{5,6}. To examine this question in the laboratory, we have taken advantage of the ability of the glass transition to freeze the structural equilibrium of a high-pressure melt and preserve it for subsequent examination in ambient conditions. Glasses of albite composition have been prepared by quenching melts under pressures of 0-80 kbar (0-8 GPa). The Al³⁺ coordination has been determined by ²⁷Al solid-state NMR spectrometry. We find that the 4-coordinated state is retained to pressures well beyond 30 kbar. A new peak at -16 p.p.m. relative to Al(H₂O)₆³⁺, which we associate with octahedral Al³⁺ appears weakly at 60 kbar and becomes a prominent feature of the spectrum at 80 kbar.

† Present address: Department of Earth Sciences, Faculty of Science, Ehime University, Matsuyama, 790 Japan.

§ Permanent address: Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, USA.

Before proceeding, we should point out that the 'fluctuations' about the average structure, which are characteristic of the liquid state, are also preserved during the high-pressure quench. This means that ambient pressure studies of the glass by small angle X-ray scattering⁷, or by equivalent techniques⁸, may yield another quantity much sought by geochemists, namely, the liquid silicate compressibility, κ_T . This is because frozen-in density fluctuations $\Delta\rho$, rather than composition fluctuations Δc , will in general dominate the scattering⁹. The compressibility is obtained via the well-known statistical thermodynamic relation, $\kappa_T = -(\Delta\rho)^2 V_m / \rho^2 RT$ (ref. 10) and we hope to report such measurements soon.

For this study, albite was synthesized by heating the mixture of reagents Na_2CO_3 , $\text{SiO}_2 \cdot x(\text{H}_2\text{O})$, and $\text{Al}_2\text{O}_3 \cdot x(\text{H}_2\text{O})$, at 1,100 °C for 20 h at 1 atm. The sample was ascertained to be a pure single phase of albite by means of X-ray diffraction. Glass samples were synthesized at various pressures of 1 atm, 20, 30, 60 and 80 kbar. The glass sample at 1 atm was prepared by heating the crystalline albite at 1,350 °C for 30 min and by quenching with a cooling rate $\sim 10^3 \text{ deg s}^{-1}$.

A piston-cylinder apparatus was used to synthesize albite glass at pressures of 20 and 30 kbar. Graphite was used for the furnace material and a Pt/(Pt + 10% Rh) thermocouple was used to measure the temperature. Crystalline albite, which was inserted into a Pt capsule, was heated to 1,500 °C at 20 kbar and to 1,600 °C at 30 kbar for 5 min, and glass was obtained by quenching the charge isobarically by switching off the heater power. The cooling rate was estimated to be $\sim 0.5 \times 10^3 \text{ deg s}^{-1}$.

Glass samples at pressures of 60 and 80 kbar were synthesized using a multiple-anvil high-pressure apparatus recently constructed at the Australian National University, the details of which are given elsewhere¹¹. The heater material was again graphite, and a (W + 5% Re)/(W + 25% Re) thermocouple was used to measure the temperature. Crystalline albite, added to graphite or boron nitride capsules, was heated above the melting temperature (1,650–1,850 °C at 60 kbar, $\sim 2,000$ °C at 80 kbar) for about 1–2 min, after which the charge was quenched by cutting the power as before. The cooling rate was estimated to be $\sim 1 \times 10^3 \text{ deg s}^{-1}$. The melting periods used should be well in excess of those needed to obtain structural equilibrium in a melt formed from a single-phase solid in which only local relaxation occurs on melting. Even at the glass transition temperature T_g , the structural relaxation time (which scales with the viscosity) is $\sim 1 \text{ min}$ ¹² and, at its melting point, the viscosity of albite is six orders of magnitude less than it is at T_g . (Some highly viscous melts nevertheless seem to retain some memory of the crystal structure from which they are derived insofar as they nucleate the parent crystal more easily than expected from long-time melting data, though we have no evidence for this in the present case.)

Despite the higher cooling rates for the high-pressure samples, it was difficult to avoid crystallization completely in the latter cases. Crystallized regions containing jadeite occurred at the sample ends where the cooling rate was smallest. These were identified under the microscope and removed before spectra were taken. One sample was deliberately crystallized to obtain a jadeite (6-coordinated Al) standard.

Because the volume of sample obtainable at 60 and 80 kbar was so small, the products of three separate preparations were combined to obtain the NMR spectra. Even so, the total mass of sample on which the 80-kbar spectrum was obtained was only 23 mg, a marginal quantity. To obtain the spectrum displayed in Fig. 2 for this sample, we had to collect and average 700,000 individual scans, requiring a total instrument time of 13 h.

NMR spectra were obtained on a Bruker CXP 300 spectrometer using the samples as synthesized (usually fragmented) without further treatment. Glass samples were suspended in powdered KBr in the Andrew type spinner. The KBr was useful for checking the spinning angle^{13,14}. Samples can easily be separated from the KBr when desired by dissolving the KBr in water.

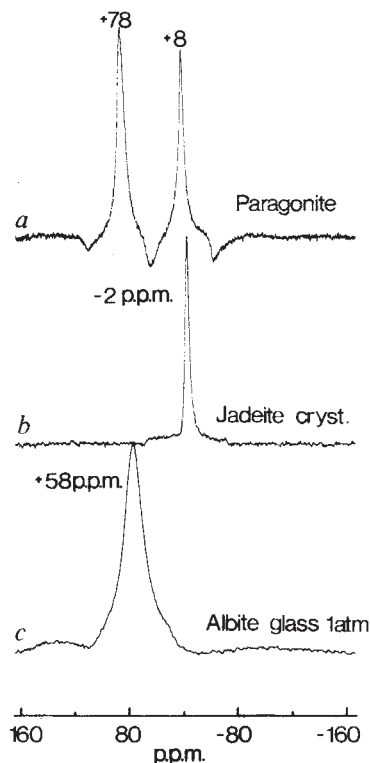


Fig. 1 ^{27}Al -NMR spectra in a, crystalline paragonite ($\text{NaAl}_2(\text{Si}_3\text{AlO}_{10})(\text{OH})_2$); b, jadeite ($\text{NaAlSi}_2\text{O}_6$); and c, albite ($\text{NaAlSi}_3\text{O}_8$), showing positions of the resonance for Al^{3+} in tetrahedral and octahedral sites referenced to $\text{Al}(\text{H}_2\text{O})_6^{3+}$ in aqueous solution.

The ^{27}Al spectra were recorded at 78.2 MHz in a field of 7 T in the Fourier transform mode. The sweep width was set to 30,000 Hz and memory size to 8 K. The usual $\pi/6$ pulses of 4 μs were applied and 10,000 free induction decays (FIDs) were collected with a repetition rate of 100 ms. A dead time of 200 μs was chosen to avoid any contribution from Al metal from the probe. The rotation rate was chosen to be 2,500 Hz (32 p.p.m.) to keep rotation bands outside the range of interest, but still inside the spectrum (as is well exemplified by the paragonite spectrum (see Fig. 1a)). Aluminium chemical shifts were measured relative to a solution of 1 M $\text{Al}(\text{NO}_3)_3$ in 1 M HNO_3 .

^{27}Al chemical shifts are known to be very sensitive to the Al coordination state¹⁴ and hence make an excellent probe for structural changes. Mostly, the ^{27}Al resonances in different coordination states fall into two distinct regions with almost no overlap. We illustrate this in Fig. 1 using aluminosilicates of known structure that are related to the structure of albite.

In paragonite, $\text{NaAl}_2(\text{Si}_3\text{AlO}_{10})(\text{OH})_2$ (a Na-mica), 33% of the Al^{3+} occurs in tetrahedral sites, 66% in octahedral sites. The latter appear to contribute less intensely to the spectrum (because of broadening by the second order quadrupole interaction) and no quantitative information can be extracted presently from peak areas or intensities. The tetrahedral Al^{3+} resonance occurs at 78 p.p.m. and the octahedral peak at +8 p.p.m. relative to $\text{Al}(\text{H}_2\text{O})_6^{3+}$. In crystalline jadeite ($\text{NaAlSi}_2\text{O}_6$) (which was here prepared by melt crystallization at 60 kbar), all Al^{3+} ions are in octahedral sites, and the resonance occurs at -2 p.p.m. (Fig. 1b).

As Müller *et al.* have pointed out¹⁵, the ^{27}Al chemical shifts are sensitive to the second nearest-neighbour coordination shell, the tetrahedral spectrum being the more sensitive to this effect. Thus, albite glass, with tetrahedrally coordinated Al^{3+} (Fig. 1c), has its resonance at 58 p.p.m., 20 p.p.m. downfield from the equivalent paragonite band. For jadeite, where Al^{2+} is octahedrally coordinated, the shift from the equivalent paragonite band is only 10 p.p.m. In each type of site in paragonite, Al^{3+} evidently experiences a higher electron density than in jadeite or albite,

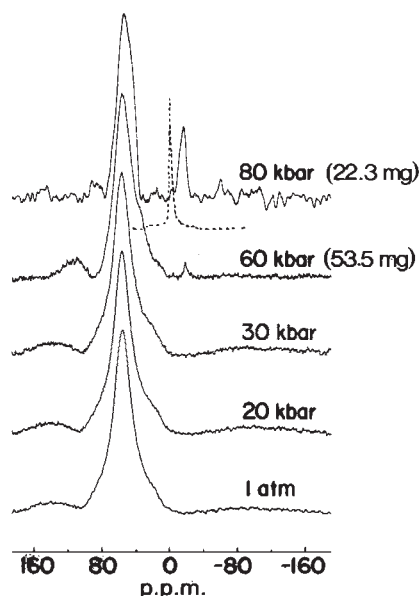


Fig. 2 ^{27}Al -NMR spectra of glassy albite samples cooled from the melt at normal pressures (lower spectrum) and at 20, 30, 60 and 80 kbar, as marked. All spectra have been normalized to the same tetrahedral peak intensity. The spectrum of ^{27}Al in jadeite (scaled to half size) is included as a dashed curve for comparisons of chemical shift with the glass octahedral component. The latter is broader than the unscaled jadeite spectrum by $\sim 50\%$ at half height, but somewhat narrower than octahedral Al in ambient pressure phosphate glasses¹³.

which is presumably caused by a greater separation of the Al^{3+} from the between-sheet charge-compensating alkali cation in the mica. The best illustration of the spectral sensitivity to such second-neighbour effects may be seen from the study of natural feldspars containing a variety of charge-compensating cations that give rise to multiple tetrahedral site resonances, one for each type of cation, separated by ~ 5 p.p.m. (our unpublished results). Site distortions due to variations in local topology in a glass may be expected to produce a similar though continuous spread of resonance frequencies.

The spectra for the normal and high-pressure albite glasses are collected in Fig. 2. Because of its importance in interpreting the 80-kbar glass sample spectrum, the jadeite spectrum is also included in Fig. 2 (light-dashed peak). It is clear that no changes in the coordination or even the site symmetry of Al^{3+} in albite glass occur under pressure increases of up to 30 kbar at least. Indeed, the spectra, once normalized to the same peak intensity, can be superimposed almost within the noise level. The persistence of this spectrum over such a pressure range implies that octahedrally coordinated Al^{3+} ions are probably not to be found in any ambient pressure oxide glasses⁴ unless the oxide ions being coordinated are pre-polarized by powerful species such as P(V) ¹³ or Re(VII) . Our results are also consistent with the absence of evidence for octahedral Al^{3+} in an earlier Raman spectral study of jadeite glass vitrified under pressures up to 40 kbar.³ In the absence of coordination changes, the compression of liquid albite must be accomplished by decreases in the Al-O-Si and Si-O-Si bond angles. The compression involves rotation of the tetrahedral packing units to permit closer oxygen packing, as described earlier for the liquid state compaction of SiO_2 itself¹⁶. This will occur more easily for adjacent AlO_4 tetrahedra and $\text{SiO}_4/\text{AlO}_4$ pairs than for $\text{SiO}_4/\text{SiO}_4$ pairs because of the smaller cation-cation repulsion involved. It seems consistent with such effects that the diffusion coefficients of trivalent cations should rise more rapidly with compression than those of tetravalent cations, as shown by Kushiro's elegant inter-diffusion experiments on jadeite-type couples¹⁷.

Unfortunately, we do not have samples in the range 30–60 kbar. It seems that somewhere in this range the tetrahedral

AlO_4 units have begun to suffer some distortion (note the shoulder on the 60-kbar spectrum), but the factors governing the line shape in ^{27}Al -NMR are sufficiently complex that we are presently unable to specify the nature of the distortion. ('Fingerprinting' with crystalline samples of known tetrahedral distortions and also with 5-coordinated Al^{3+} will be described elsewhere.) Together with the increased distortion is the simultaneous appearance of a small peak of -16 p.p.m., which rises above the noise level in the 60-kbar spectrum. In the 80-kbar sample spectrum, this new band has risen to almost half the height of the residual tetrahedral spectrum. Comparison with the paragonite spectrum suggests that a considerable fraction of the Al^{3+} is now in octahedral coordination. In the 80-kbar spectrum the tetrahedral spectrum is narrower and indeed its full width at half height is somewhat smaller than in the glass prepared in ambient conditions.

The new band at -16 p.p.m. is evidently not attributable to residual unseparated jadeite, the spectrum of which peaks at -2 p.p.m. (see Fig. 2). Only jadeite and co-crystallized coesite were detected in the X-ray examinations (and removed, see above), so the new band seems unlikely to be attributable to crystalline material, despite its narrowness. The possibility that it is a metastable jadeite precursor phase distributed in crystallites too small (< 100 Å) to give detectable X-ray lines, although unlikely, cannot be eliminated without performing annealing studies on the high-pressure glass. These will be performed once the glass has been fully characterized. Al_2O_3 , an alternative possible phase in high-pressure crystallization conditions, has a resonance in the range $+4$ – 0 p.p.m. according to our examination of samples from eight different sources, and hence cannot explain our results.

The simplest interpretation of our observations is that the initial effect of very high pressure ($P > 40$ kbar) on the framework liquid is a distortion of the tetrahedral symmetry around Al^{3+} (note the shoulder, Fig. 2), which is then relieved at higher pressure ($P > 60$ kbar) by the splitting off of a tightly defined subgroup of octahedrally coordinated Al^{3+} ions, the concentration of which will depend on the applied pressure.

These observations are not inconsistent with the results of ion dynamics computer simulation studies on liquid jadeite^{5,6}, which showed that both Al and Si coordination numbers could be changed with application of sufficient pressure to the high-temperature liquid. The change occurred at lower pressures for Al^{3+} ('midpoint' at about 100 kbar) than for Si (midpoint at ~ 200 kbar). However, because of the high temperature that had to be maintained in these computations (for structural equilibrium to be reached within acceptable computation times), distinct coordination states were not discernible; the midpoint corresponded to an average coordination of five.

The observations are also qualitatively consistent with predictions based on the assumption of structural similarities between melt and the crystalline phase with which it is in equilibrium (quasi-crystalline melt model). For instance, jadeite only occurs at the albite liquidus above 32 kbar pressure at $\sim 1,430^\circ\text{C}$ ¹⁸, so both 20- and 30-kbar samples, in which Al is exclusively in 4-coordination (Fig. 2), would have albite on the liquidus. The $6 \rightarrow 4$ equilibrium is presumably dependent on temperature, and it should be remembered that the equilibrium being frozen is that which exists at the glass transition temperature, T_g , which usually is at 0.6 – $0.7 T_m$. Because the albite/jadeite boundary on the T/P phase diagram has a positive slope, the quasi-crystalline model evidently overestimates the tendency towards octahedral coordination of Al in the melt. It suggests, however, that the 6-coordinated state of Al is more easily generated at the jadeite composition although a poorer resistance to crystallization, probable at this composition, could hamper its observation by the present 'preserve-and-look' approach.

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ESR dating of volcanic ash

Noboru Imai & Koichi Shimokawa

Geological Survey of Japan, 1-1-3 Higashi, Yatabe, Tsukuba, Ibaraki, Japan

Michio Hirota

Meteorological Research Institute, 1-1 Nagamine, Yatabe, Tsukuba, Ibaraki, Japan

Radiation-induced centres of Al, Ti, Ge and E' occurring in the natural quartz of various geological materials were discussed a decade ago by McMorris^{1,2}; but it is only recently that the peroxy centre for natural flint³, the E' centre for quartz from a fault⁴ and the Ge centre for quartz from welded tuff⁵ have been used for electron spin resonance (ESR) dating. The ages of volcanic ash or lava have been obtained by thermoluminescence (TL) dating using fine (2-11- μm) volcanic glass⁶ or plagioclase feldspar⁷, respectively. However, ESR dating of volcanic ash has proved extremely difficult because signal intensities of radiation-induced centres measured at room temperature are weak compared with the interfering signals from paramagnetic impurities such as Fe^{3+} and Mn^{2+} . Here we have used a signal from a quartz aluminium centre, measured at 77 K, where it is several orders of magnitude greater than those of the E' or Ge centres at room temperature. The aluminium-centre ESR signal can also be observed in plagioclase feldspar and volcanic glass, allowing the age of the volcanic debris to be determined using any of the three mineral compounds.

The method was tested in three samples whose ages had already been obtained by other methods. Quartz was extracted from Tamagawa rhyolite welded tuff (TM1) in Hachimantai (north-east of Japan), plagioclase from Aira-Tn ash (TTS34) of Kokubu in Kagoshima Prefecture (south of Japan) and volcanic glass from volcanic ash (TE5) in the Ohiso hills (middle of Japan) or from the upper portion of the Takio Formation in the Ohita Group (south of Japan). Quartz grains, 2-3 mm in diameter, from the Tamaga welded tuff were hand picked after crushing the rock and then cleaned by 3 M HCl. The grain was crushed gently and sieved to obtain 0.25-0.125-mm particles. Volcanic glass and plagioclase feldspar were isolated by heavy liquid separation (with specific gravities of 2.5 and 2.8) after the sample has been washed in water, sieved to 0.25-0.125 mm and cleaned both ultrasonically and with 3 M HCl. Each sample was examined microscopically and found to be of greater than 90% purity. For the ESR measurement, 200-mg samples of

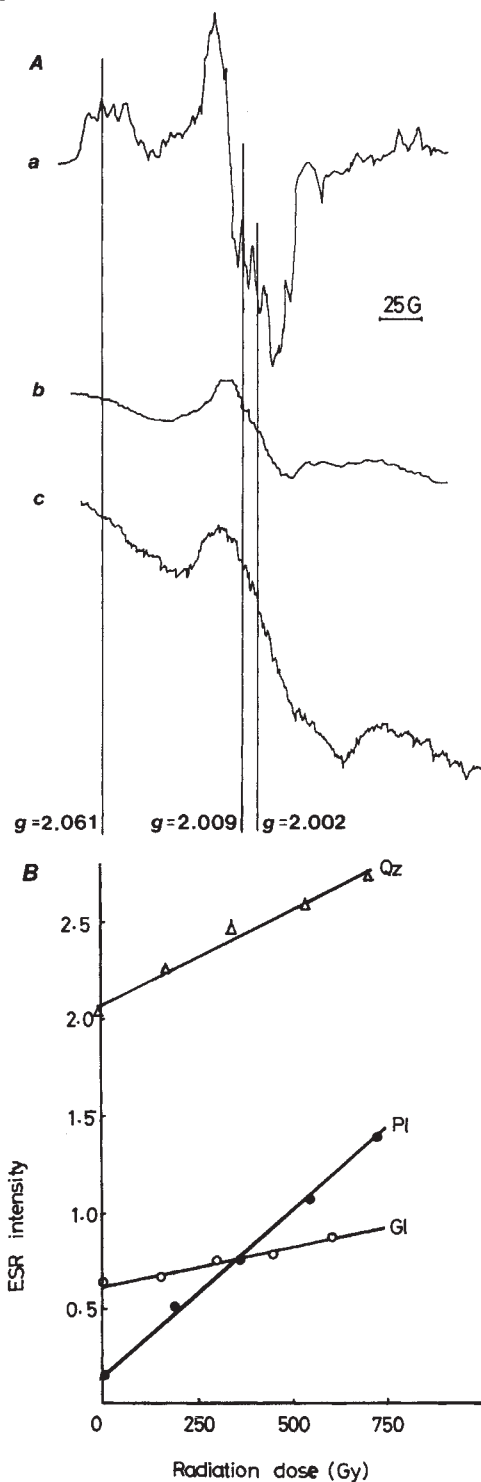


Fig. 1 A, The ESR spectra of Al centre in natural quartz TM1 (a), plagioclase TTS34 (b) and volcanic glass TE5 (c). The principal values of the g tensor of this centre¹¹ are shown. B, The relation of signal intensity with radiation dose for quartz (Δ), plagioclase ($\bullet$) and volcanic glass ($\circ$).

volcanic glass and 100-mg samples of quartz and plagioclase were used. An X-band spectrometer (JEOL FE) was used at 77 K with 100-kHz field modulation. Artificial γ -irradiation was carried out with a ^{60}Co source. Measurements were repeated five times with the same sample so that average values could be obtained. The concentration of uranium and thorium was determined by spectrophotometry using arsenazo III and torin, respectively, after solvent extraction of tri-n-octylphosphine oxide (TOPO)/cyclohexane^{8,9}. The potassium content was determined by atomic absorption spectrometry¹⁰.

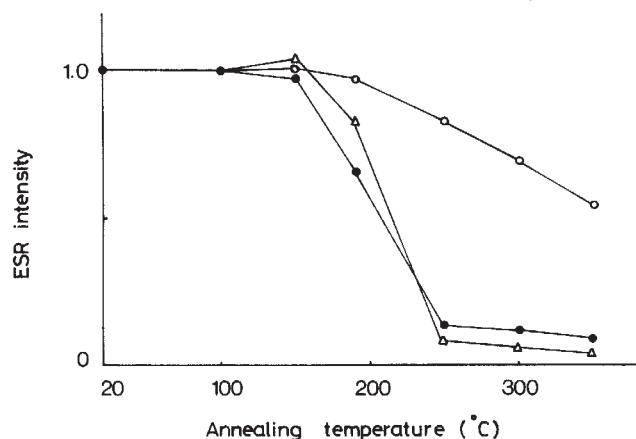


Fig. 2 Thermal annealing of Al centre in quartz TM1 (Δ), plagioclase TTS34 ($\bullet$) and volcanic glass TE5 ($\circ$). Annealing temperature was maintained for 15 min in each step.

Figure 1A shows the ESR spectra of the aluminium centre in natural quartz, plagioclase and volcanic glass. An aluminium centre is a defect in which an aluminium ion replaces the silicon site and a hole is trapped at one of the four ligand oxygens. Schnadt and R  uber reported the ESR spectrum of this defect in quartz powder and fused glass¹¹. The signal is measured at 77 K and broadens beyond the detection at room temperature in quartz powder. The signal intensity in plagioclase and volcanic glass is several times smaller because of extra line broadening due to a more distorted crystal field or to a looser lattice structure.

We have investigated the thermal annealing of the Al centre. Isothermal experiments were performed at several temperatures for quartz (TM1), plagioclase (TTS34) and volcanic glass (TE5) and the mean lifetimes of the traps were estimated to be 1.8–3.8 Myr, ~ 4 Myr and 10^3 Myr, respectively, at 10 °C. The frequency factor was assumed to be $3 \times 10^{12} \text{ s}^{-1}$ and trap depth was 2.4 eV for quartz and plagioclase and 2.8 eV for volcanic glass. The changes of the ESR signal for quartz, plagioclase and volcanic glass on successive heating (for 15 min) are shown in Fig. 2. The quartz and plagioclase signals almost disappeared above 250 °C, whereas that of volcanic glass changed only slightly at that temperature, indicating that the aluminium centre in volcanic glass is much more stable. Moreover, above 250 °C, in plagioclase and volcanic glass, a large peroxy-centre signal appeared in the middle of the aluminium centre signal.

Figure 1A shows the ESR spectra of the aluminium centre the irradiation dose. The total dose was obtained by extrapolating the least-square-fitting line. The radiation for the minerals is assumed to be the sum of the internal α radiation and the external β and γ radiations considering the grain size of 0.25–0.125 mm. The annual dose was calculated from the analyses of the internal and bulk contents of uranium, thorium and potassium using Bell's data¹². The α -efficiency, or k factor, is tentatively assumed to be 0 for quartz⁴, 0.20 for plagioclase⁷ and 0.04 for volcanic glass¹³. The internal and bulk uranium, thorium and potassium concentrations, annual dose and ESR age are shown in Table 1. For our calculation the internal α dose for TTS34 plagioclase was taken as only 6%, because the internal contents of uranium and thorium are very small, whereas that for TE5 volcanic glass is 11%. In addition to this, the external α dose (the size effect) gives an error of 7% for plagioclase and 0.6–1.4% for volcanic glass assuming a 10% external α -dose contribution¹³. However, these were not included in the calculation.

The ESR age of quartz samples (TM1) was 1.13 Myr, which agrees with the fission track age of 1.2 Myr (ref. 14). The ESR age of plagioclase of TTS34 was 2.8×10^4 yr. The ¹⁴C age of widespread tephra of Aira-Tn (AT) ash has been determined to be $2.1\text{--}2.2 \times 10^4$ yr (ref. 15). (Note, however, that ages of $2\text{--}10 \times 10^4$ yr were obtained for volcanic glass samples of the AT ash from several different locations.) The ESR age of the volcanic glass of TE 5 is 0.34 Myr which agrees well with the fission track age of 0.39 Myr obtained using zircon¹⁶. This agreement encouraged us to apply the method to a suite of volcanic ashes of unknown ages.

The ESR ages of six volcanic ash layers in the Takio Formation of the Ohita Group were obtained using volcanic glass, as shown in Table 2. The concentration of uranium, thorium and potassium varies in each bed and, hence, the annual dose varies from 1.69 to 3.89 Gy yr⁻¹. However, the ESR age increases from 0.49 to 0.82 Myr in agreement with the stratigraphical sequence. The volcanic ash in this area has been correlated with the upper part of the Ohsaka Group (0.3–0.8 Myr)¹⁷.

Another possible use of ESR is for 'fingerprinting' the ash layers. The ESR spectra of the aluminium centre in the AT ash from different locations have the peroxy-centre signal in the middle of aluminium-centre signal. In our work, two out of three samples of plagioclase and five out of eight samples of volcanic glass have peroxy-centre signals. In other volcanic ash, none of the 15 samples of plagioclase and only two out of 28 samples of volcanic glass exhibit this signal, which may thus be unique to AT ash. In the annealing experiment, the peroxy-centre appears above 250 °C for plagioclase and volcanic glass, which suggests the mechanism by which this signal is formed.

Table 1 Radioactivity data, total dose and ESR ages for samples of known age

Mineral	U		Th		K (%)	Annual dose (Gy yr ⁻¹)	Total dose (Gy)	ESR age (Myr)	Age from other work (Myr)
	(bulk p.p.m.)	(internal p.p.m.)	(bulk p.p.m.)	(internal p.p.m.)					
Quartz (TM)	1.2	—	4.0	—	1.48	1.96	2,210	1.13 $\pm$ 0.22	1.2 $\pm$ 0.3 (ref. 14)
Plagioclase (TTS34)	2.6	0.2	6.9	0.75	2.44	3.64	102	0.028 $\pm$ 0.004	0.021–0.022 (ref. 15)
Volcanic glass (TE5)	2.2	2.0	6.7	10.9	3.85	5.10	1,680	0.33 $\pm$ 0.05	0.39 $\pm$ 0.08 (ref. 16)

Table 2 Total dose and ESR age of volcanic ash of upper part of Takio Formation of Ohita Group

Depth (m)	U		Th		K (%)	Annual dose (mGy yr ⁻¹)	Total dose (Gy)	ESR age (Myr)
	(bulk p.p.m.)	(internal p.p.m.)	(bulk p.p.m.)	(internal p.p.m.)				
1	4	0.8	1.0	2.5	6.3	2.45	1,410	0.49
2	6	1.0	0.4	4.2	4.0	1.04	840	0.50
3	7	1.8	1.0	2.3	4.4	1.16	1,050	0.54
4	15	1.3	1.6	3.3	6.7	2.05	1,870	0.66
5	30	1.2	0.8	4.9	6.0	1.40	1,620	0.73
6	35	1.3	1.2	6.4	6.7	3.02	3,150	0.82

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Oxygen isotope fractionation between amorphous silica and water at 34–93 °C

Itsuro Kita*, Sachihito Taguchi† & Osamu Matsubaya*

* Research Institute of Underground Resources, Mining College, Akita University, Tegata Gakuen-cho, Akita 010, Japan

† Research Institute of Industrial Science, Kyushu University, Kasuga, Fukuoka 816, Japan

The fractionation factor of oxygen isotopes between quartz and water has been extensively studied from experimental^{1,2}, theoretical^{3,4,5} and empirical viewpoints⁶ and has been widely used to estimate the isotopic composition or the temperatures of water relating to various geological processes. At lower temperatures, where hydrothermal processes, diagenesis and other processes take place, however, the fractionation factor for quartz as well as other silica minerals has not been studied thoroughly. We report here experimentally determined oxygen isotope fractionation factors between amorphous silica and water at 34–93 °C, obtained by measuring the oxygen isotope ratios of amorphous silica precipitated from geothermal waters of power plants. The relationship between the fractionation factor and temperature is similar to the extrapolated relationship of quartz observed at higher temperatures. This result is useful for the oxygen isotope study of water–rock interactions at temperatures below 200 °C.

The amorphous silica was precipitated at four points (P-1, P-2, P-3 and P-4) along the reinjection lines in the Otake and Hatchobaru geothermal power plants. Although it would have been preferable to obtain the amorphous silica at many points over a wide range of temperatures, these four points were the only ones available for this experiment. The same geothermal

water from the Otake station flows at P-1 and P-4; at P-2, the geothermal waters from both stations are mixed; at P-3, the geothermal water is from the Hatchobaru station. The temperatures of flowing geothermal waters are 93, 87 and 81 °C at P-1, P-2 and P-3, respectively. The temperature at P-4 gradually decreased from 70 °C to atmospheric temperature during this study because of silica scaling in the pipeline. Samples of amorphous silica were collected at 58 and at 34 °C.

The differences in the chemical character and roughness of surface of the materials on which amorphous silicas were precipitated may influence the oxygen isotope fractionation factor between amorphous silica and water. Materials such as wire, stainless steel tubing, glass tubing, rock, wooden rod and Teflon plate were immersed in the geothermal waters at P-1 and P-4. At P-2 and P-3, only wire was used. The materials were immersed in the geothermal waters for about two weeks at P-1 and P-3, and one month at P-2 and P-4. During these periods, the geothermal waters were collected at least once in a day and their temperatures, oxygen isotope ratios, and Cl[−] and total silica contents were measured. At P-2, however, these measurements were done at intervals of a week. The temperatures and oxygen isotope ratios of geothermal waters fluctuated within the errors shown in Table 1.

The pH, and Cl[−] and total silica contents are 8.3, 1,680 p.p.m. and 525 p.p.m., respectively, at P-1, and 6.9, 2,870 p.p.m. and 730 p.p.m., respectively, at P-3. The fraction of ionic silica in the total silica was also determined. The ionic silica is determined using a conventional molybdate yellow method for an aliquot of geothermal water of which the pH was adjusted to ~1.2–1.5 just after collection. Most of the total silica is ionic at P-1, whereas about 90 and 55% is ionic at P-2 and P-3, respectively. This is mainly because of the differences in pH and the time elapsing after the geothermal water was separated from the steam^{7,8}. The content of ionic silica was not measured at P-4.

The precipitated silica did not contain any crystalline materials which could be detected by X-ray diffraction, except for the samples at P-1 which contain kaolinite of < 5 wt%. From the results of chemical analysis of silica scales at the geothermal plants⁹, the precipitated silica seems to have impurities of less than a few per cent. Such a small amount of contamination does not significantly affect the oxygen isotope ratio of amorphous silica.

The oxygen isotope ratios of amorphous silica and geothermal water were measured using a fluorination¹⁰ and an H₂O–CO₂ equilibration method, respectively. The isotope ratios were expressed as $\delta^{18}\text{O}$ values relative to the SMOW standard. The $\delta^{18}\text{O}$ of silica was referred to SMOW on the basis of a fractionation factor of 1.0407 between CO₂ and H₂O (ref. 11). The $\delta^{18}\text{O}$ value of NBS-28 is +9.36‰ in this method.

Table 1 $\delta^{18}\text{O}$ values of amorphous silicas precipitated from geothermal waters on various materials and for the geothermal waters, and the fractionation factor between amorphous silica and water

Sampling point	Temperature (°C)	Date	Materials	$\delta^{18}\text{O}_{\text{SMOW}}$ (‰)		Fractionation factor (α)
				Amorphous silica	Geothermal water	
P-1	92.7 ± 0.3	August 1983	Iron wire	15.2	−5.4 ± 0.1	1.0207
			Wooden rod	15.6		1.0211
			Glass tubing	15.3		1.0208
P-2	87 ± 3	February 1983	Iron wire	17.9	−5.2 ± 0.2	1.0231
P-3	80.8 ± 1	March 1983	Iron wire	20.9	−4.3 ± 0.1	1.0253
P-4	58 ± 10	December 1982	Iron wire	22.7	−5.4 ± 0.1	1.0283
			Glass tubing	23.1		1.0287
			Teflon plate	22.8		1.0284
			Rock	23.0		1.0286
			Stainless steel tubing	23.1		1.0287
P-4	34 ± 5	February 1983	Iron wire	27.5	−5.4 ± 0.1	1.0331

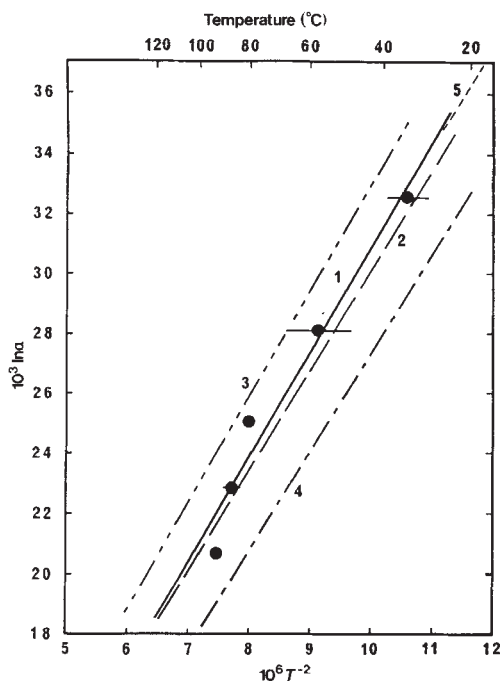


Fig. 1 Comparison of the oxygen isotope fractionation factor between amorphous silica and water with those previously reported for quartz. ●, Present results. Line 1, the least square fitting in the present study. Line 2, from refs 1, 2. Line 3, from Taylor (cited in ref. 3). Line 4, from ref. 4. Line 5, from ref. 12.

The dehydration of hydrous silica changes the oxygen isotope ratio of the silica remaining⁶. In this study, however, air-dried amorphous silicas were preheated in vacuum at 200 °C for 3 h before the reaction with fluorine gas, for the following reasons.

The SiO₂ content calculated from the amount of oxygen extracted by fluorination ranged from 88 to 95 wt%. These reductions (5 to 12 wt%) in content correspond to the weight loss when the amorphous silicas were heated at 1,000 °C. Therefore, these reductions must be due to dehydration of silica during preheating. In that case, the water in the amorphous silica can be completely removed at 200 °C, and much of the water should be removed at temperatures below 200 °C. The water removed at such lower temperatures may be simply adsorbed water, or water weakly combined with silica; the dehydration causes very little change in the oxygen isotope ratio of silica remaining¹².

As shown in Table 1, the amorphous silicas precipitated on the different materials both at P-1 and at P-4 have the same $\delta^{18}\text{O}$ value at each point. This indicates that the difference in the character of surface of the materials does not influence the oxygen isotope fractionation between silica and water. Scanning electron microscopy confirms that the difference in the character of surface has no effect on the morphology of amorphous silica.

The fractionation factors (α) between amorphous silica and geothermal water are given by

$$\alpha = (1 + 10^{-3} \delta^{18}\text{O}_{\text{silica}}) / (1 + 10^{-3} \delta^{18}\text{O}_{\text{water}})$$

as shown in Table 1. The relationship of $\ln \alpha$ to reciprocal temperature square is almost linear, as seen in Fig. 1, and is approximated by

$$10^3 \ln \alpha = 3.52(10^6 T^{-2}) - 4.35$$

This relationship is similar to that of the equilibrium fractionation factor between quartz and water. It is especially close to the extrapolated relationship between quartz and water as determined experimentally by Clayton *et al.*¹ and Matsuhisa *et al.*².

Knauth and Epstein⁶ estimated the fractionation factor between quartz and water to be 1.039 at 0 °C using the observed oxygen isotope ratio of a microcrystalline quartz in an oceanic sediment. This value is close to the relationship determined by

Kawabe⁴ and lower by about 5% than the present result. Labeyrie's¹² measurement of the fractionation between silica in sponges and diatoms and water is very close to the present one.

An interesting problem is whether the oxygen isotope fractionation takes place among polymorphs of silica. In the case of α and β quartz, for instance, fractionation of 0.8 to 1.5‰ is theoretically predicted^{3,4}, although no evidence has been found from experimental studies². In the case of amorphous silica, the problem is whether a definite fractionation factor is available, because amorphous silica has no definite crystal structure. Nevertheless, the present result suggests that the extent of fractionation between amorphous silica and quartz may be smaller than the extent of variation in the equilibrium fractionation factors reported in the literature for the quartz-water system.

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Major Asian aeolian inputs indicated by the mineralogy of aerosols and sediments in the western North Pacific

Marsha Blank*, Margaret Leinen†
& Joseph M. Prospero*

* Rosenstiel School of Marine and Atmospheric Science,
4600 Rickenbacker Causeway, Miami, Florida 33149-1098, USA
† Graduate School of Oceanography, University of Rhode Island,
Kingston, Rhode Island 02881, USA

Recent atmospheric chemistry studies have shown that substantial quantities of soil material are being transported out of Asia and across large regions of the North Pacific^{1,2}. In the central North Pacific, the estimated¹ ($6-12 \times 10^{12} \text{ g yr}^{-1}$) and measured² ($20 \times 10^{12} \text{ g yr}^{-1}$) deposition rate of mineral aerosols could account for a substantial fraction of the non-biogenic portion of deep-sea sediments in this region, where sedimentologists have previously estimated that 75-95%^{3,4} of the surface sediment is derived from atmospheric dust fallout. The quantitative importance of wind-transported (aeolian) material to sedimentation in the North Pacific would be more firmly established if it could be shown that the composition of the present-day soil aerosol is similar to that of mineral particles in the underlying sediments, which have been deposited over a period of thousands of years. Here we compare the compositions of soil aerosols and sediments collected from the western North Pacific and find that their mineralogies are identical, except for one mineral. This supports the hypothesis that the sediments are heavily impacted by aeolian sources.

The mineralogy of the $<2\text{-}\mu\text{m}$ size fraction of both aerosols and sediments was determined by X-ray powder diffractometry using methods developed specifically for $<2\text{-}\mu\text{m}$ sediments⁵. This size fraction should yield a substantial fraction of the total aerosol mass, which has a median diameter of $\sim 2\text{ }\mu\text{m}$ ^{6,7}. Aerosols of this size have a very small settling velocity in the atmosphere; hence, they will have a relatively long residence time and their composition will be little affected by changes due to differential settling of different-sized mineral constituents.

The sediment samples were taken from the tops of 12 piston cores (Table 1b) collected during R/V *Vema* cruise 36-12 (Fig. 1). Core sites are located on the seaward side of the western Pacific trenches in an area where there is no evidence of turbidites⁸ or of inputs from ice rafting⁹. On the basis of their locations, mineralogy and stratigraphy, it has been suggested that the inorganic (non-biogenic) mineral component of the sediments is predominantly aeolian in origin^{10,11}. Although there are differences in sedimentation rates between the areas sampled by coring, there is no evidence of extensive reworking of sediments by bottom currents or erosion at the sites¹². Finally, the average age of the sediments used for this study is < 10 kyr¹³. Thus, they probably have not been influenced by the major climatic changes associated with Pleistocene glaciations, with the possible exceptions of the three cores 33P, 45P and 49P, which show ages of 12–17 kyr¹³.

The mineral aerosols were collected with two 1-m² nylon mesh samplers¹⁴ aboard the DV *Glomar Challenger* during the Deep-Sea Drilling Project (Table 1a) in the western North Pacific (Fig. 1). Of the samples collected in this region, the six largest were selected for analysis. Previously, there has been concern that the collection efficiency of the meshes may vary with particle size so that the samples would not be representative of the total aerosol¹⁴⁻¹⁶. In a recent study, however, the <2- μ m mineralogies of soil aerosols collected concurrently with filters and with meshes were found to be identical except for smectite, which was underestimated in the mesh samples relative to the filter samples by a factor of about two (M.B. and J.M.P., in preparation).

Sediment samples were treated with sodium acetate (buffered to pH 4.8 with acetic acid) to remove CaCO_3 , with 0.4 M Na_2CO_3

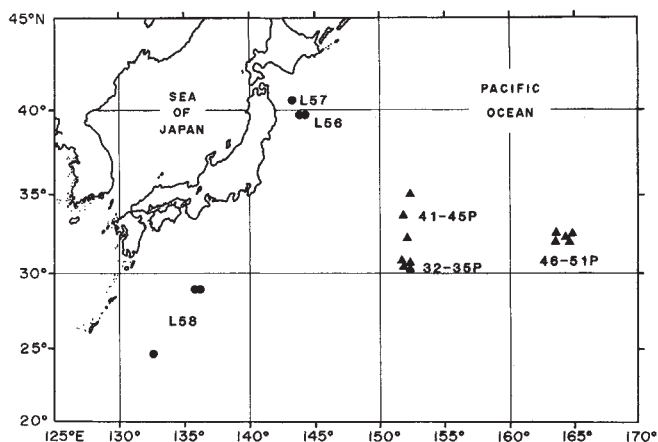


Fig. 1 Collection sites of *Glomar Challenger* aerosol samples and Vema 36/12 32P-51P sediment cores. The circles and triangles represent the aerosols and sediments, respectively. (See Table 1.)

to remove biogenic silica (opal), and with a Na dithionite/Na citrate solution to remove amorphous iron and manganese oxides¹⁷. Subsequent treatment and analysis was identical for both aerosol and sediment samples. Bulk aerosol sample masses ranged from 0.4 to 4.3 g per sample. Part (200–500 mg) of each sample was saturated with 1.0 M MgCl_2 (ref. 5). Size separations were made by wet-sieving at 20 μm diameter. The $<2\text{-}\mu\text{m}$ fraction was isolated by a settling procedure. To increase the precision of X-ray analysis, an internal standard of 10 wt% $<2\text{-}\mu\text{m}$ talc was added to each $<2\text{-}\mu\text{m}$ sample⁵, which was then deposited as a slurry on a 0.45- μm silver filter and X-irradiated⁵ (see Table 3). The peak areas for illite, smectite, kaolinite, chlorite, quartz and plagioclase were converted to wt% using the weighting factors determined by Heath and Pisias⁵ for modern North Pacific sediments. Amphibole concentrations were undetectable or present in only trace amounts. The sum of the wt% of the major mineral components is equivalent to the percentage of diffracting phases in the sample. The average

Table 1 Aerosol and sediment samples

Aerosol mesh samples collected aboard the <i>Glomar Challenger</i>					
Leg	Sample Mesh	On site	Date sampled	Sampling period (h)	Ship location
56	2	434B	23-24 September 1977	25.5	39°45' N 144°06' E
56	3	434B	27-28 September 1977	30.5	39°45' N 144°06' E
57	1	438A	22-24 October 1977	52.3	40°38' N 145°33' E
58	2	442	16-18 December 1977	47.0	28°59' N 136°04' E
58	3	442A	18-20 December 1977	50.2	28°59' N 136°04' E
58	17	446	20-22 January 1978	47.5	24°42' N 132°47' E

Piston core samples from <i>Vema</i> 36-12 cruise				
Univ. Rhode Island accession no.	Core	Depth (cm)	Latitude	Longitude
58,752	V36/12-32P	6-8	30°55.3' N	151°37.1' E
58,753	V36/12-33P	10-12	30°20.7' N	152°16.4' E
58,754	V36/12-34P	10-12	30°45.6' N	152°16.2' E
58,755	V36/12-35P	8-10	30°30.1' N	151°48.7' E
58,756	V36/12-41P	10-12	32°21.1' N	152°03.1' E
58,757	V36/12-43P	10-12	33°45.0' N	151°45.8' E
58,758	V36/12-45P	12-14	35°05.6' N	152°16.4' E
58,759	V36/12-46P	3-5	32°41.1' N	163°31.7' E
58,760	V36/12-47P	2-4	32°06.0' N	164°32.0' E
58,761	V36/12-48P	3-5	32°08.9' N	163°28.6' E
58,762	V36/12-49P	8-10	32°24.0' N	164°16.0' E
58,763	V36/12-51P	2-4	32°38.0' N	164°46.0' E

Average water depth, 5,100 m (ref. 8).

Table 2 Analytical variability caused by machine fluctuations and laboratory methods

	Qtz	Plag.	Smec.	Illite	Kaol.	Chl.	I/Q	K/C	I/K
$\bar{x}$	8.8	10.1	0.8	42.0	15.5	3.2	4.81	4.84	2.77
s.d.	0.52	1.68	0.64	2.35	2.15	0.27	0.47	0.87	0.51
r	8.2-9.4	7.5-12.2	0.2-2.0	37.7-45.6	12.3-18.8	2.9-3.6	4.14-5.56	3.61-6.06	2.01-3.51

Aerosol sample L58K3 (<2- μ m size fraction) was analysed eight times. Data are normalized to 80.4% diffracting. Qtz, quartz; Plag., plagioclase; Smec., smectite; Kaol., kaolinite; Chl., chlorite; I/Q, illite/quartz ratio; K/C, kaolinite/chlorite ratio; I/K, illite/kaolinite ratio; $\bar{x}$, mean; r, range.

Table 3 Comparison of mean composition of <2- μ m sample size fraction

	Qtz	Plag.	Smec.	Illite	Kaol.	Chl.	I/Q	K/C	I/K
Aerosols ($n = 6$)									
$\bar{x}$	10.5	11.2	1.1	39.5	15.5	2.7	3.81	5.81	2.66
$s_{\bar{x}}$	0.49	0.86	0.26	1.92	1.16	0.15	0.29	0.38	0.29
Sediments ($n = 12$)									
$\bar{x}$	9.9	9.7	3.1	38.7	16.4	3.0	3.90	5.72	2.61
$s_{\bar{x}}$	0.27	0.41	0.44	0.89	1.18	0.15	0.15	0.49	0.24

Samples were X-irradiated on a Phillips Norelco X-ray diffractometer using Ni-filtered $\text{CuK}\alpha$ radiation at 40 kV and 25-40 mA. Samples were scanned over the range 3-30° (2 θ) at a rate of 1 deg min⁻¹. Weight percentages are normalized to maximum percentage diffracting, 80.4%.

percentage of diffracting phase in the aerosols was 55.7% and that for the sediments was somewhat higher, 71%, because more amorphous component was removed before X irradiation. The residual fraction (100-% diffracting) is composed of poorly crystalline minerals and X-ray amorphous phases in the sample³. To facilitate the comparison of the different samples, the mineral percentages were normalized to the maximum diffracting percentage, 80.4%.

The variability in X-ray clay mineral analysis can be very high because the analyses are extremely sensitive to sample preparation and instrumental effects. Multiple runs were performed of many samples to assess the analytical precision and, in one case, an aerosol sample was subsampled and analysed eight different times to determine the error due to sample preparation and day to day variability in the X-ray diffractometer. The precision is good with the exception of smectite (Table 2).

The mineralogies of the mesh samples and the sediments are strikingly similar (Table 3). The differences in composition between the two materials are less than the analytical variability except for smectite. Statistical *f*-tests and Student's *t*-tests indicate that the aerosols and sediments are statistically identical (99% confidence level) except for smectite. This difference for smectite is probably not significant, because of the relatively low precision in smectite analysis, the small percentages involved and the possible undersampling of smectite by the mesh samplers. The amount of biogenic opal and other non-terrestrial components in the bulk sediment ranges from 2-20% in the area of our cores¹⁰, which, with the above evidence, suggests that the remaining 80-98% of the sediment is aeolian in origin and that aeolian inputs dominate the deep-sea sedimentation there. The location of our samples, atmospheric haze data¹⁸ and the results of North Pacific dust trajectory studies (J. T. Merrill *et al.*, in preparation) suggest that the arid regions of Asia are the source of the mineral aerosol.

The rate of mineral aerosol deposition in this region seems quite high. The total dust deposition rates measured recently at island stations 3,500-5,600 km east of the Vema core locations are 0.064 g cm⁻² kyr⁻¹ at Midway and 0.043 g cm⁻² kyr⁻¹ at Oahu¹. Average sedimentation rates for the cores vary from 0.86 cm kyr⁻¹ for the cores near 152° E to 0.67 cm kyr⁻¹ for those near 164° E¹³ and dry sediment bulk densities average 0.3-0.4 g cm⁻³ for both areas¹⁹. If ~80% of this material is aeolian, the average mass accumulation rates of aeolian material for the two core areas are 0.24 g cm⁻² kyr⁻¹ and 0.18 g cm⁻² kyr⁻¹. These

higher rates may be attributable to the fact that the Vema sites are closer to the Asian sources where dust fluxes could be substantially higher. We have estimated a mean dust concentration for the core locations using that measured in the Sea/Air Exchange (SEAREX) programme's North Pacific island network^{2,6} and Asian dust concentrations measured in a similar network in Japan (M. Uematsu, personal communication). A semilogarithmic interpolation between these networks yields a mean mineral aerosol concentration of 6-10 $\mu\text{g m}^{-3}$. On the basis of deposition models developed in the SEAREX studies¹, we estimate the mineral aerosol deposition rate at the core sites to be 0.2-0.3 g cm⁻² kyr⁻¹, in good agreement with the measured aeolian sediment accumulation rates. These results suggest that the present rate of dust transport out of Asia is representative of transport over the past few thousand years. It follows that the non-biogenic mineral components in North Pacific sediments could be a good indicator of palaeoclimatic conditions such as aridity, wind velocity and large-scale atmospheric circulation patterns.

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Thermogenic hydrocarbons in surface sediments of the Bransfield Strait, Antarctic Peninsula

M. J. Whiticar*, E. Suess† & H. Wehner*

* Bundesanstalt für Geowissenschaften und Rohstoffe, Stilleweg 2, 3000 Hannover, FRG

† College of Oceanography, Oregon State University, Corvallis, Oregon 97331, USA

Antarctica is the only continent from which petroleum deposits have not been encountered, despite the occurrence of potential hydrocarbon basins onshore and around its margins. We have discovered thermogenic hydrocarbons in unconsolidated Recent sediments from the King George Basin, Bransfield Strait, west Antarctica. These sediments possessed a marked petroliferous smell throughout the length of the 8.6-m gravity core. The basin sediments are glacial marine deposits dominated by turbidites and contain abundant autochthonous organic matter as a result of high seasonal primary productivity in this most fertile part of the circumpolar ocean¹. The maturation of this material into hydrocarbons may have been accelerated by the high geothermal gradient in the basin, associated with back-arc spreading created by the subduction of the Drake Plate into the South Shetland trench². Sediments in this rifted basin are frequently intruded by volcanic sills and dykes³ giving rise to acoustic features shown in Fig. 1. The presence of these thermogenic hydrocarbons provides the first demonstration of active source rocks in Antarctica.

On core retrieval, the liners were immediately cut into 1-m sections and ~500 g of wet sediment removed to a freezer and kept at -80 °C until analysed (within 24 h). The analytical procedure for the molecular and stable isotope composition of the hydrocarbon gases is similar to that given elsewhere⁴. The bitumen fraction (C_{15+}) was extracted with CH_2Cl_2 by soxhlet, partitioned by liquid chromatography (LC) and analysed by gas chromatography/mass spectrometry⁵. Interstitial waters were pressure filtered immediately after coring. Total dissolved carbonate (ΣCO_2) was determined by gas chromatography following acidification and stripping of the sample and dissolved sulphate (SO_4^{2-}) was determined gravimetrically.

Methane yields are low in the uppermost 3 m (< 32 parts per 10^9 wt gas/wt sediment) then increase to a maximum of 153 parts per 10^9 at 450 cm (Fig. 2). The distribution of ethane to pentane hydrocarbons (C_{2+}) roughly covaries with methane, with a maximum of 197 parts per 10^9 in the sediments (Table

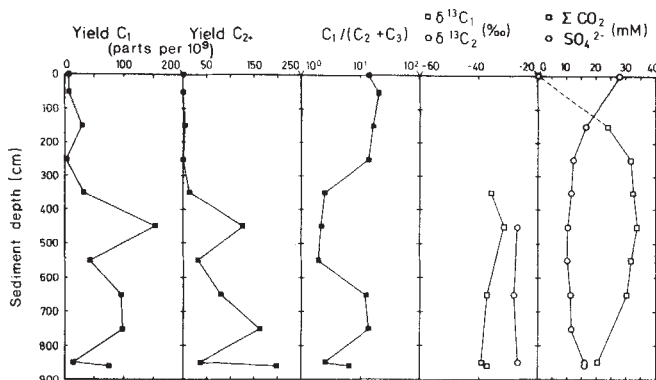


Fig. 2 Hydrocarbon gas concentrations of methane (C_1) and ethane through pentane (C_{2+}) increases with sediment depth in core 1140. Thermogenic gas signature confirmed by $C_1/(C_2+C_3)$ and by $\delta^{13}C_1$ and $\delta^{13}C_2$ values in the thermal range. The presence of sulphate throughout the core precludes *in situ* biogenic methane production. Remineralization of organic matter appears to cease below 300 cm, possibly owing to lower TOC contents (<0.9%).

1a). In addition, some of the gas chromatograms from the deeper samples reveal C_6 and C_7 hydrocarbons in concentrations even greater than those of C_1 - C_5 . Unsaturated hydrocarbons, ethene and propene were present in only minor amounts in comparison to the *n*-alkanes. Within the C_{2+} fraction, the concentration tends to increase corresponding to carbon number below 650 cm (Table 1b). Relative to methane the C_{2+} gas fraction is greater at depth, with % wetness ratios ($[\Sigma C_{2-5}/\Sigma C_{1-5}] \times 100$) reaching values of 39%. The high % wetness here is related to hydrocarbon formation via thermal processes, rather than via diagenetic processes which may produce minor quantities of C_{2+} (ref. 6).

Methane produced microbially is frequently observed in strongly reducing sediments, with associated minor C_{2+} concentrations 2-4 orders of magnitude lower. Several other locations in the Bransfield Strait exhibit bacterial methanogenesis in the sediments, but only under strongly reducing conditions where the interstitial dissolved sulphate has been exhausted. Dissolved sulphate in core 1140-1 showed evidence of reduction, but SO_4^{2-} persisted in large amounts throughout the core (Fig. 2), precluding bacterial methanogenesis as a source of the methane. In core 1140-1, methane contents are lower and C_{2+} contents much higher than those found in other sediments of the King George Basin exhibiting bacterial methanogenesis.

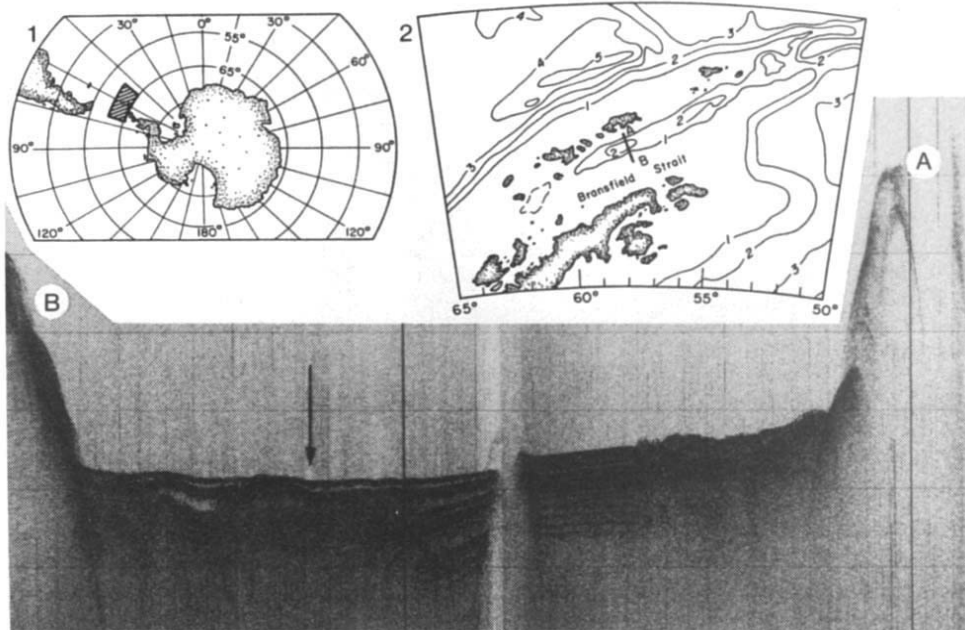


Fig. 1 Section of 3.5-kHz sediment profile showing northern (A) and southern (B) limit of King George Basin. Inset 1 is a polar projection of Antarctica showing the investigation area; inset 2 gives the bathymetry (1,000-m isobaths) and 3.5-kHz track between King George Island and the Antarctic Peninsula. Core 1140 (indicated by arrow) was taken in fine-grained turbid sequences of hemipelagic muds overlying an acoustic feature where loss of signal occurs below 5-m sediment depth. The steep basalt intrusion north of the station can be seen (centre of photo).

Table 1 Hydrocarbon gas and interstitial fluid analyses from core 1140-1, King George Basin

<i>a</i>							
Depth (cm)	Yield C ₁ (parts per 10 ⁹)	Yield C ₂₊ (parts per 10 ⁹)	C ₁ /(C ₂ +C ₃)	δ ¹³ C ₁ (‰)	δ ¹³ C ₂ (‰)	[SO ₄ ²⁻] (mM)	ΣCO ₂ (mM)
0	6	0	25.9	ND	ND	ND	ND
50	8	0	38.4	ND	ND	ND	ND
150	29	2	33.8	ND	ND	16.13	23.5
250	3	0	25.5	ND	ND	11.86	31.3
350	32	13	6.1	-35.8	ND	11.24	32.2
450	153	125	4.7	-31.4	-26.8	10.11	33.5
550	43	31	4.1	ND	ND	9.77	31.4
650	96	80	27.4	-37.1	-28.0	10.95	29.7
750	99	162	28.9	ND	ND	11.31	ND
850	15	36	5.2	-39.0	-26.8	15.68	20.1
860	76	197	14.5	-37.1	ND	15.68	ND

<i>b</i>							
Depth (cm)	C ₁	C ₂	C ₃	% Hydrocarbons iso-C ₄	n-C ₄	iso-C ₅	n-C ₅
0	94.1	3.6	0.0	0.0	0.0	0.0	0.0
50	97.4	2.5	0.0	0.0	0.0	0.0	0.0
150	96.8	2.1	0.7	0.0	0.0	0.0	0.0
250	96.2	3.7	0.0	0.0	0.0	0.0	0.0
350	85.9	4.9	9.1	0.0	0.0	0.0	0.0
450	76.1	12.1	4.2	1.9	1.5	2.6	0.7
550	76.5	13.2	5.3	1.7	1.1	0.8	0.2
650	81.7	1.8	1.1	2.7	2.8	6.6	2.5
750	71.6	1.8	0.6	0.9	1.4	15.4	7.5
850	60.6	9.5	2.0	2.9	2.3	11.2	11.2
860	61.3	2.4	1.7	3.4	2.7	15.5	12.6

ND, not determined.

Carbon isotope ratios of methane and ethane were measured on the samples with higher yields and are reported in the usual δ notation relative to the PDB standard. Methane in the deeper core sections is slightly more depleted in ¹³C (-37.1 to -39.0‰, Fig. 2) than in those nearer the surface (-31 to -35‰). Carbon isotopes of ethane were more uniformly distributed between -26.8 and -28.0‰. Thermal maturities of natural gases can be estimated using the relationship of carbon isotope ratios of methane and ethane to vitrinite reflectance^{7,8}. Based on such relationships (M.J.W. and E. Faber, in preparation), the inferred maturity of the gases in the deeper core section ranges between 1.4 and 1.8% *R*_o (equivalent) for methane and between 1.5 and 1.7% *R*_o (equivalent) for ethane (Fig. 3). The good agreement between them supports the idea that the methane and C₂₊ hydrocarbons in these sediments have a similar source and thermal history. The average maturity estimate of 1.6% *R*_o (equivalent) of the gases places them in a late thermal stage, possibly in the condensate range.

A combination of the molecular and carbon isotope composition in the classification diagram of Bernard *et al.*⁹ (Fig. 4) shows the gases to be of the late thermal associated type. The possibility of an admixture of an overcooked dry methane with an associated wet gas can be discounted because of the agreement in maturity between the C₁ and C₂ isotope data. There is no evidence of secondary alteration effects such as anaerobic oxidation in the deeper sediments. Although the low methane concentrations in the uppermost sediments may have been influenced by oxidation, the expected associated relative enrichment in C₂₊ was not observed.

The composition of solvent extracts further indicates the presence of thermal hydrocarbons in these surface sediments. The five extracted sediment samples yielded 82–320 p.p.m. (dry wt) soluble material (Table 2), which is relatively high for Recent sediments with 0.62% and 0.87% total organic carbon (TOC). The total extract/total organic carbon ratio (mg extract/g C_{org}) increases from 13 at the surface to 46 at the bottom. The lower three samples had sufficient extract for LC partitioning of which more than 50%, hetero-components and other polar compounds, was irreversibly bound to the LC column. Nevertheless, hydrocarbon concentrations are found up to 141 p.p.m. at 860 cm, which is somewhat anomalous for this diagenetic setting.

The mixture of hydrocarbons from a deeper more mature source with autochthonous bitumen is expressed most clearly in the samples from 350 and 650 cm. The saturated C₁₅₊ *n*-alkanes are dominant between C-numbers 25 and 31, apart from the deepest sample (860 cm) where phytane constitutes the major component. Where detectable, phytane is more concentrated than pristane signifying a reducing depositional environment¹⁰ consistent with the interstitial water chemistry. *n*-Alkanes show an odd-even predominance (OEP) of 1.32–1.50 (Table 2), excepting the sample at 350 cm where the OEP is 1.04.

There is a relatively high contribution of rearranged steranes at 650 cm, which would not be expected in immature Recent sediments. Furthermore, the sediment from 350 cm possesses a hopane signature characteristic of many oils. In particular, the ratio of the 17- α (H)-hopane, C₃₁ 22S/22R, is > 1 at 350 cm (Table 2). With these exceptions, the extracts are typical of

Table 2 Analysis of C₁₅ hydrocarbons from core 1140-1

Depth (cm)	TOC (%)	Extract (p.p.m.)	Saturated (p.p.m.)	Aromatic (p.p.m.)	Heterocyclic (p.p.m.)	OEP	tr/st	Ts/Tm	C ₂₉ -20S/C ₂₉ -20R	C ₃₁ -22S/C ₃₁ -22R
5	0.62	82	ND	ND	ND	1.32	5	0.53	0.43	0.64
150	0.85	150	ND	ND	ND	1.57	7	0.36	0.21	0.67
350	0.87	257	84	40	108	1.04	5	0.81	0.67	1.17
650	0.85	189	17	35	114	1.51	4	0.54	0.43	0.47
860	0.70	320	44	98	113	1.41	1	0.11	ND	0.43

OEP, odd-even predominance (*n*-alkanes); tr/st, triterpane/sterane ratio; Ts/Tm, 18 α (H)-trisanorhopane/17 α (H)-trisanorhopane. ND, not determined.

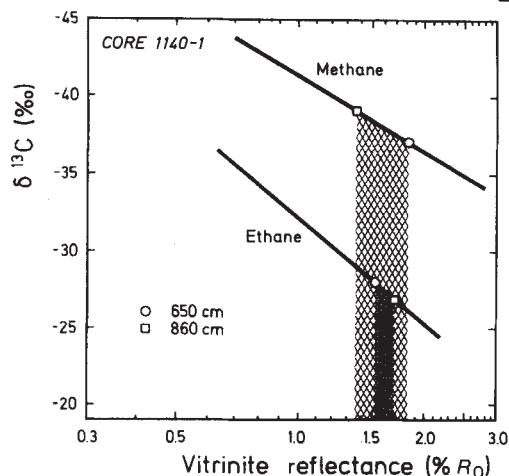


Fig. 3 Maturity of source organic matter generating the hydrocarbon gases is estimated using the empirical relationship between $\delta^{13}\text{C}$ values of C_1 and C_2 and maturation levels (M.J.W. and E. Faber, in preparation). These gases are formed in the range of the late-mature stage corresponding to a mean vitrinite reflectance of $\sim 1.6\%$ R_0 equivalent.

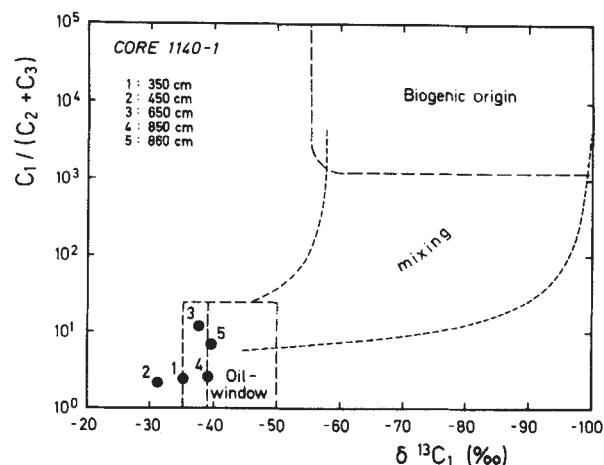


Fig. 4 Natural gas genetic classification diagram after Faber *et al.*²⁵ clearly showing late-stage thermal character of the hydrocarbon gases measured. There is no evidence for biogenic or mixed gases in these cored sediments.

immature organic material, with contributions of terrestrial organic material, shown by the OEP, of the higher carbon number (C_{24+}) n -alkane, and low contents of rearranged steranes (5- β sterane is less abundant than 5- α steranes and 20S-ethylcholesterane is less than 20R-ethylcholesterane). In addition, the Ts/Tm triterpane ratios (18- α (H)/17- α (H)-trisnorhopane¹¹) of these samples are < 1 , the $\text{C}_{29} + \text{C}_{30}$ moretane contents are relatively high and C_{31} -22S of 17- α (H)-hopanes is less than C_{31} -22R (Table 2). Thus, although the degree of impregnation is somewhat low in samples at 350 and 650 cm, there are positive indications that heavier thermogenic hydrocarbons have migrated into these sediments.

The maturity of the migrated hydrocarbon component in these samples is roughly estimated by the OEP and biomarkers to be early mature ($< 0.5\%$ R_0 equivalent). The discrepancy between the maturity of the light hydrocarbon gases as determined by carbon isotopes (1.6% R_0 equivalent) and that of the allochthonous C_{15+} hydrocarbons based on biomarkers ($< 0.5\%$ R_0 equivalent) and the *in situ* vitrinite reflectance ($< 0.2\%$ R_0) would suggest that the gases come from more mature organic sources possibly more deeply buried than those generating the heavier bitumens. Such gases could assist in the transport of the bitumens acting for example as a solvent (compare ref. 12). The limited number of cores makes it difficult to locate the source of the hydrocarbons, or to define their regional surface expression. Coring attempts at a second station nearby (1185-1) were frustrated by basalt, but analyses of a small sample of sediment and basaltic debris remaining in the core catcher showed clear traces of C_{2+} hydrocarbon gases.

The thermogenic hydrocarbons were cored in acoustically-turbid horizons in the sediments of the eastern edge of the basin (Fig. 1). The cause of the acoustic turbidity is unknown. The measured light gas concentrations are not even close to the solubility limit necessary to form free gas bubbles¹³ and the heavier hydrocarbons (C_{5+}) are liquid at the *in situ* pressure and temperature. In addition, the sediments cored are within the stability zone of gas hydrates¹⁴. If the gas contents were high enough to permit a free gas phase, this gas would be incorporated into a hydrate structure, though it is possible that gases at depths greater than those of the cored sediment may be trapped beneath, or in, hydrate caps¹⁵. Such preferential removal, or trapping, of the light (C_1 - C_4) gases could be one explanation for the relatively high C_{5+} contents in the surface sediments. There were, however, no indications direct or indirect, of hydrates in this acoustically-turbid zone in the 3.5-kHz trace (Fig. 1). Such seismic indicators of gas hydrates are not always visible and because of the shallow coring depth (860 cm, maximum) the possibility of hydrates cannot be disregarded.

Another explanation for the absence of reflectors would be the presence of oligostromes or mud diapirs, which frequently occur in areas of rapid subsidence with undercompaction and greater heat flow. Upward-transported sediments or fluids could carry the hydrocarbon gases and even bitumens acquired at greater depth¹⁶; vertical fluid expulsion or flushing (mentioned above) could take place in the area of coring resulting in the decrease in bitumen and higher relative molecular mass bias ($> \text{C}_{24+}$) close to the surface, despite no change in organic facies or maturity; the lower methane concentrations in the uppermost sediments could likewise be affected. Unfortunately, surface morphology and sedimentology do not substantiate this explanation.

The King George Basin is a tectonically active zone which has extensional features such as normal dip-slip faults and basalt intrusions located close to station 1140-1 (Fig. 1, centre); higher geothermal gradients would be expected in such a geological setting. The Guaymas Basin is a comparable tectonic setting with a high geothermal gradient where hydrocarbons have been thermally generated from mature organic matter in younger than normal sediments in the proximity of intrusions¹⁷.

Previous offshore drilling along Antarctic continental margins has been limited to Leg 28 of the Deep Sea Drilling Project in the Ross Sea and off the Shackleton Ice Sheet¹⁸. At three sites in the Ross Sea, minor amounts of ethane and higher homologues were measured along with substantial quantities of methane¹⁹. Carbon isotope data reported for the methane (-68 to -79%) and the strong sulphate reduction at these sites^{18,20} would indicate microbial methanogenesis. The contribution from thermogenic sources in this region, based solely on the molecular composition data, is uncertain. Recently, investigations on the opposite side of Antarctica, off Wilkesland, have reported bacterial gas in low concentrations, but no thermogenic gas was indicated²¹.

Hydrocarbon seeps are common for most of the world's major petroleum accumulations many of which are also trapped in graben structures as in the Bransfield Strait²². The large-scale rift faults and igneous intrusions can create migration pathways to the surface for gaseous and liquid hydrocarbons. Several analogous cases have been reported, as for example, in California, Mexico, Sumatra and numerous locations extending along the Andes from Columbia to Terra del Fuego^{23,24}. The hydrocarbon seepage in core 1140-1, however, is not on the same scale as those seeps and thus should be regarded as a micro seepage. Nevertheless, the data provide unambiguous geochemical evidence of active petroleum source rocks along the Antarctic continent.

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Somatostatin immunoreactivity in neuritic plaques of Alzheimer's patients

John H. Morrison*, Joseph Rogers†, Stephen Scherr*, Robert Benoit‡ & Floyd E. Bloom*

* Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines, La Jolla, California 92037, USA

† Department of Neurology, University of Massachusetts Medical Center, 55 Lake Avenue, North, Worcester, Massachusetts 01605, USA

‡ Department of Medicine, McGill University, Montreal General Hospital Research Institute, 1650 Cedar Avenue, Montreal, Canada H3G 1A4

Senile dementia of the Alzheimer's type can be diagnosed with certainty only by examining neurofibrillary tangles and neuritic plaques under the microscope¹⁻⁴. Recently, it has been suggested that the condition is linked to specific neurotransmitter systems⁵, with a decline of cortical acetylcholine, choline acetyltransferase⁶⁻⁸, cholinergic neurones projecting to the cortex⁹⁻¹¹, cortical noradrenergic content¹², locus coeruleus neurones^{13,14} and cortical somatostatin content¹⁵. Using immunocytochemical methods¹⁶, we here report that somatostatin-immunoreactive processes are present in neuritic plaques in human Alzheimer's specimens¹⁷. These data, as well as other reports of non-cholinergic changes^{18,19}, strongly imply that Alzheimer's disease cannot be linked exclusively to cortical cholinergic elements, as proposed previously⁵. Rather, our data on plaque and somatostatin co-localization and distribution patterns suggest that Alzheimer's neuropathology may involve primarily the loss of selective cortical neurones that are targets of the implicated transmitter systems and that plaque formation may result from the degeneration of presynaptic and postsynaptic neurites of large projection neurones in layers III and V. Given the neurochemically heterogeneous input to these cells, it is not surprising that several neurotransmitter systems, one of which is somatostatin, are implicated in the pathology of Alzheimer's disease.

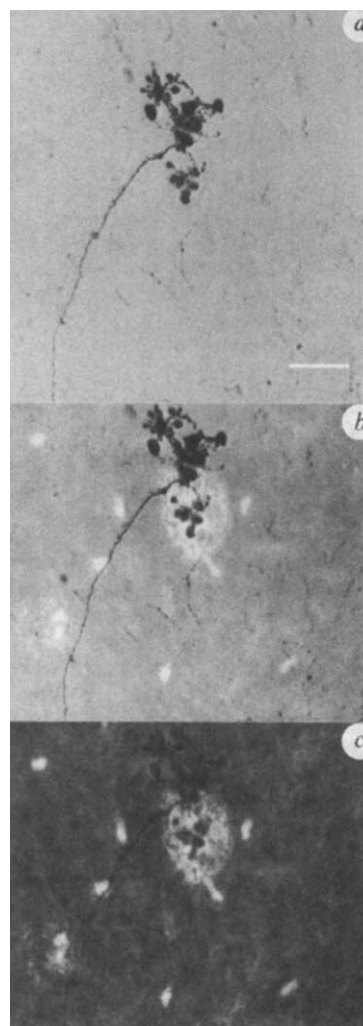


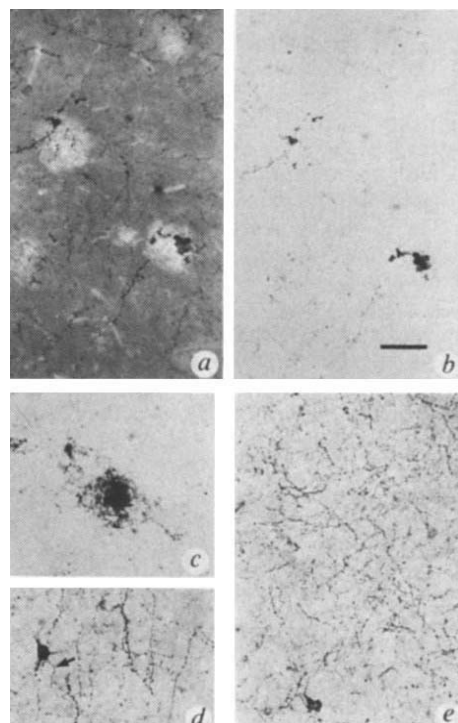
Fig. 1 Somatostatin immunoreactivity and a thioflavin-S-labelled neuritic plaque in an Alzheimer's patient: *a*, *b* and *c* describe the same microscopic field, representing the combined visualization of fluorescently labelled neuritic plaques (thioflavin-S) and somatostatin-immunoreactive profiles seen in conventional bright-field optics. *a*, Photomicrograph of this particular field in full bright field; *b*, the image obtained when viewed with fluorescence optics combined with low-intensity bright field; *c*, full fluorescence image alone. The somatostatin-immunoreactive profile (black) seems to be a cluster of degenerating boutons, many of which are bulbous and distended. In *b* and *c*, much of this pathological profile is associated with a fluorescent, amyloid-containing plaque with no clear core and thus possibly relatively immature^{23,24}. Also, note that some of the bulbous profiles are outside the amyloid portion of the plaque. This complex may represent an early transitional stage where the plaque has not yet reached its full extent. Scale bar, 40 μ m.

The present experiments examined normal adult, aged adult and Alzheimer cortices using antibodies against somatostatin-28 and somatostatin-28(1-12) (ref. 20). Specific cortical regions from postmortem samples of five clinically and neuropathologically confirmed Alzheimer brains and four brains from elderly patients with no neurological deficits were analysed. In some cases, sections were immunohistochemically stained to localize the prosomatostatin-derived peptides and then counterstained with thioflavin-S, a fluorescent stain that visualizes neuritic plaques and neurofibrillary tangles²¹. All Alzheimer brains contained neuritic plaques and neurofibrillary tangles in all regions stained.

In the human cortex, somatostatin antisera specific for either somatostatin-28 or somatostatin-28(1-12) show regional and laminar patterns similar to those observed in rodent cortex¹⁶, but with a greater density of innervation. Somatostatin-positive

Fig. 2 Somatostatin immunoreactivity in human cortex. *a, b*, The same microscopic field in layer III of a section through the pre-central gyrus of a postmortem sample from an Alzheimer's patient. This section and *c* were stained immunohistochemically with S320 and counterstained with thioflavin-S. *a*, A fluorescence photomicrograph with the section exposed to fluorescence optics and low-intensity bright-field optics simultaneously. In this photograph, the fluorescent thioflavin-S-positive plaques show up as white puffs, somatostatin-immunoreactive fibres as darkly stained profiles. *b*, Exposed only to bright-field optics, thus the fluorescent plaques are not visible, but the immunoreactive diaminobenzidine-containing profiles are. Note that both of the large white plaques are associated with bulbous somatostatin-immunoreactive profiles and in both cases a labelled fibre can be seen entering the periphery of the plaque. *c*, Photomicrograph of a section stained with S320 through the superior frontal gyrus of a different Alzheimer's patient from that shown in *a* and *b*. Note the large coalescence of tangled somatostatin-immunoreactive fibres which have the appearance of a neuritic plaque; when viewed with fluorescence optics, this profile is not fluorescent and thus presumably does not contain amyloid. *d, e*, Photomicrographs of sections stained with 320, from postmortem samples of the frontal lobe of a neurologically normal 82-yr-old female. In *d*, the arrow points to a putative axon emerging from a labelled cell in layer III tentatively identified as a basket cell^{16,26}. *e*, Normal density of somatostatin-immunoreactive fibres in layers II and III of frontal lobe from the same patient. Note the presence of highly arborized, varicose fibres. The cortical regions studied were the superior frontal gyrus, parahippocampal and inferior temporal gyrus, superior temporal gyrus, anterior cingulate gyrus, precentral gyrus, post-central gyrus and striate cortex.

Methods. Human tissue was obtained 4–16 h postmortem, immersed in fresh 4% paraformaldehyde phosphate buffer (pH 7.4) for 5 days, followed by graded sucrose solutions (12–18%) for several days and then sectioned by cryostat (40- μ m sections). The sections, pretreated in 1% hydrogen peroxide for 15 min to remove intrinsic peroxidase activity¹⁴, were stained immunohistochemically as reported previously¹⁶, with antisera 309 and 320, immunochemically characterized previously²⁰. S309 is directed against the N-terminal 12 amino acids of somatostatin-28 and thus recognizes both somatostatin-28 and somatostatin-28(1–12). The antigenic determinant of S320 is the C-terminus of somatostatin-28(1–12). The Glu-OH at the carboxy-terminus of the molecule is crucial for antigenicity so that neither somatostatin-28 or somatostatin-14 is read. Any N-terminal extension of somatostatin-28(1–12) will be recognized by S320 (see refs 16, 20). The tissues were then stained with thioflavin-S which has been shown to stain amyloid-containing profiles²¹. To stain with thioflavin-S, mounted tissue sections were hydrated in distilled H₂O for 10 min, followed by immersion in a filtered aqueous 1% thioflavin-S solution for 10 min. Sections were then differentiated and dehydrated through a series of alcohols (70% absolute) into xylene and coverslipped in Permount (Fisher). The tissues were viewed with a Zeiss microscope equipped for both fluorescence and bright-field optics. Scale bar in *b*, 60 μ m (applying to all photomicrographs).



cell bodies and terminals exhibit a bilaminar distribution; for example, there are few labelled cells or fibres in layer IV, but much higher densities of labelled cells in layers II, superficial III and VI. In the human cortex, the terminal field in layer I is even denser than in rat cortex; there are also labelled cell bodies in the subcortical white matter^{17,22}. Human cortex, compared with rat cortex, has a much greater degree of regional variation in density of somatostatin-positive staining. The cingulate, temporal and frontal cortices have the densest somatostatin-positive terminal arborization of all neocortical regions; parietal cortex has an intermediate density and the primary visual cortex has the least dense innervation.

The general regional and laminar patterns of somatostatin immunoreactivity for terminals and cell bodies in Alzheimer cortices were similar to those in postmortem samples from normal elderly patients with no clinical or autopsy signs of Alzheimer's disease. All Alzheimer specimens, however, contained striking evidence of direct pathological involvement of somatostatin-containing cortical neurones. Swollen bulbous profiles interpreted as degenerating somatostatin-positive fibres (Figs 1, 2*a, b*), were readily apparent. With combined fluorescence bright-field optics, immunohistochemical material counterstained with thioflavin-S further revealed that these bulbous, clustered, swollen profiles were associated often with amyloid-containing neuritic plaques. Most of the plaques containing somatostatin-immunoreactive material appeared to be relatively 'immature', with no clear amyloid core. However, 'mature' plaques with a clear amyloid core and apparently degenerating somatostatin-positive profiles at the core perimeter were also observed. In addition, non-bulbous, somatostatin-positive fibres with a normal varicose appearance were seen often to surround or end just inside the periphery of mature plaques. Depending on the region and layer examined, 30–50% of the definitive early and mature plaques contained some somatostatin immunoreactivity. This percentage is surprisingly high for co-localizations based on single sections of plaques.

Reconstruction of the entire three-dimensional extent of plaques would probably reveal an even higher proportion. Moreover, compared with perfused monkey cortex, only a fraction of somatostatin profiles are revealed in postmortem Alzheimer's material. Finally, despite lacking thioflavin staining for amyloid, other presumptively degenerating bulbous somatostatin-immunoreactive profiles could represent a stage of plaque formation where amyloid deposition is minimal, as in primitive plaques^{23,24}. Indeed, some deteriorating somatostatin-immunoreactive profiles resemble clearly the primitive plaque of classical neuropathology (see Fig. 2*c*). Profiles similar to those seen in Alzheimer cases (Fig. 2) were seen rarely in normal aged cortices. Future experiments will aim at determining whether or not a given plaque contains multiple neurotransmitter markers.

The laminar and regional distributions of Bielschowsky-stained and thioflavin-positive plaques in Alzheimer material bear a striking similarity to distributions of somatostatin-positive terminals in normal human and animal material¹⁷. Most of the plaques in Alzheimer's specimens, like somatostatin-positive terminals in normal specimens, are in the supragranular layers, particularly layer III, with layer V having intermediate density and layer IV the lowest density of plaques and somatostatin innervation. Most of the somatostatin-positive plaque complexes (see Figs 1, 2*a, b*) are in layer III, where neurone loss in Alzheimer's disease is maximal²⁵ and most of the cortico-cortical association systems originate. In addition, plaque density in Alzheimer's material and somatostatin-terminal density in normal material show an almost identical regional profile: cingulate, frontal and temporal regions have the highest density of plaques and somatostatin-immunoreactive terminals, parietal cortex has an intermediate density of both and the primary visual cortex has the lowest of both. Thus, the limbic and association areas of the cortex, which are dominated by cortico-cortical association systems rather than thalamo-cortical connections, are the areas with the highest plaque density²⁶ and somatostatin

innervation. Collectively, these laminar and regional data suggest that plaque formation and somatostatin innervation are linked to similar elements of cortical circuitry. Plaques may arise from the degeneration of a complex of the postsynaptic neurites of large layer III or V projection neurones which form the cortical association systems²⁷ and their associated inputs containing neurotransmitters such as acetylcholine, noradrenaline, somatostatin or substance P²⁸.

The combined neurochemical and neuropathological findings in Alzheimer specimens lead to the hypothesis that functional deficits may result from the breakdown of both neocortical association systems and divergent, global cortical afferents such as the cholinergic projection from the nucleus basalis and the noradrenergic projection from the nucleus locus coeruleus. Whether the degeneration of these ascending systems is primary or secondary to a primary intra-cortical pathology, the break-

down of these global afferent systems plus a breakdown of the cortico-cortical associational systems could give rise to profound loss in attention, arousal and intellectual function, with minimal effects on specific sensory or motor performance. In this view, Alzheimer's disease could represent a dissociation of the various components of those cortical functions subserved by distributed systems²⁹. The multiple transmitter systems involved in the pathological process suggest that no single transmitter can provide a therapeutic strategy and that the nature of the primary pathological insult remains the main question.

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Location of neuronal tangles in somatostatin neurones in Alzheimer's disease

G. W. Roberts*, T. J. Crow* & J. M. Polak†

* Division of Psychiatry, Clinical Research Centre, Harrow, Middlesex HA1 3UJ, UK

† Department of Histochemistry, Hammersmith Hospital, London W12 0HS, UK

Senile dementia of the Alzheimer type is a chronic, progressive neuropsychiatric condition characterized clinically by global intellectual impairment¹ and neuropathologically by the presence of numerous argyrophilic plaques and tangles²⁻⁴. Neurochemical investigations have established loss of the cholinergic^{5,6} and aminergic^{7,8} projections to the cerebral cortex and a loss of the content of somatostatin⁹⁻¹², with preservation of cholecystokinin^{11,13} and vasoactive intestinal polypeptide^{11,14}, neuropeptides also located in cells intrinsic to the cortex. We describe here the relationship between cortical somatostatin immunoreactivity and the plaques and tangles of diseased tissue by immunocytochemical and silver impregnation techniques on paraffin-embedded tissue. In sections of Alzheimer's tissue, cortical somatostatin-immunoreactive perikarya exhibited morphological changes consistent with neuronal degeneration. Silver-stained material immunostained subsequently showed that many neurones containing tangles were also somatostatin positive. No such co-localization was observed using antisera to other neuropeptides. Our findings indicate that a subclass of somatostatin-positive neurones are affected selectively in Alzheimer's disease and that these neurones also contain neuronal tangles. Thus, destruction of somatostatin-containing neurones is an early and perhaps critical event in the disease process.

Brains were obtained postmortem from six controls (4 males, 2 females, mean age 76 yr, range 58-87 yr) and 15 clinically and

neuropathologically defined cases of Alzheimer's (5 male, 10 female, mean age 77 yr, range 57-91 yr). Brains were fixed routinely in 4% formalin and blocks of tissue which included the left temporal lobe structures (temporal cortex, hippocampus, amygdala) were taken and paraffin embedded, then 20- μ m coronal sections cut for histological examination. Plaques and tangles were demonstrated using a modified Palmgren's method¹⁵, somatostatin and other neuropeptides by immunocytochemical techniques (see Fig. 1 legend for details).

In control tissue, somatostatin immunoreactive neurones were numerous in temporal and entorhinal cortex and parahippocampal gyrus (Fig. 1a). The neurones were localized in cortical layers II-III and V-VI and were predominantly of the non-pyramidal type (multipolar and bitufted; see Fig. 1b), showing a range of morphological diversity. Numerous somatostatin fibres were also observed running radially with some lateral branching towards the pial surface and down to the subcortical white matter. Many somatostatin-positive neurones were found adjacent to the subcortical white matter with their dendritic fields oriented parallel to it; fibres from these cells penetrated into the white matter, possibly to form part of an intracortical association system. Somatostatin-positive neurones and fibres were also observed in the subiculum, pro-subiculum, hippocampal CA1-4 (stratum oriens and pyramidal), the hilus of the dentate gyrus and the latero-basal, central, medial and cortical nuclei of the amygdala. Large numbers of multipolar (Golgi type II) neurones and fibres were also observed in the caudate nucleus and putamen (Fig. 1c).

On immunohistochemical examination, Alzheimer's cases showed marked decreases in somatostatin content. In the superficial layers (II and III) of the temporal cortex, parahippocampal gyrus and uncus, both neuronal and fibre staining was depleted substantially; some regions showed no observable immunoreactive neurones and had a concomitant decrease in local fibre innervation. In addition to the decreases in immunoreactive material, many somatostatin-positive cells (particularly in deeper cortical layers) were observed which showed morphological changes consistent with neuronal degeneration and

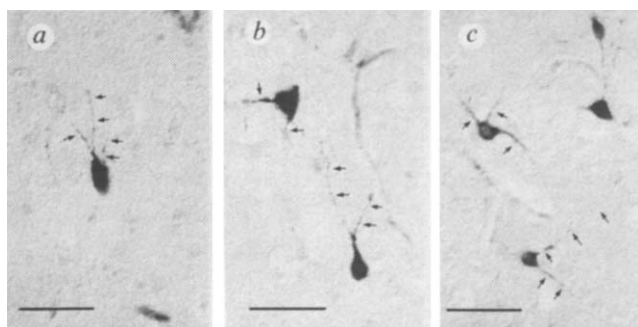


Fig. 1 Somatostatin-28(1-14 Tyr)-immunoreactive neurones in control tissue. *a*, Typical bitufted cell-type observed frequently in cortical layers II-V with dendrites oriented towards the pial surface (arrows). Different morphological subtypes of neurones are seen in the cortex. *b*, Multipolar cell with laterally extending dendrites (arrowed) in close proximity to a bitufted neurone with radially extending dendrites (arrowed) in layer III of the cortex. *c*, Example of the medium-sized aspiny (Golgi type II) somatostatin-immunoreactive interneurons found throughout the caudate. Each cell has two to four processes (arrowed) which curve as they course away from the cell body. Scale bars, 50 μ m.

Methods. Sections used for the co-localization of tangles and somatostatin immunoreactivity were processed first for silver impregnation, rinsed in phosphate buffer and processed subsequently for immunocytochemistry. For silver impregnation, sections were processed according to the protocol of Cross¹⁵. For immunocytochemistry, 20- μ m sections of tissue were incubated overnight with specific antisera raised to: somatostatin-28(1-14 Tyr) (RIA), dilution 1/1,000; somatostatin-14 (ref. 16), dilution 1/2,000; vasoactive intestinal polypeptide, dilution 1/10,000 (ref. 19); cholecystokinin-octapeptide, dilution 1/500 (ref. 19) and neuropeptide Y, dilution 1/1,000 (ref. 17) according to a previously described protocol for the peroxidase/anti-peroxidase method¹⁶. Pre-absorption of the diluted somatostatin-28(1-14 Tyr) antiserum with 50 μ g somatostatin-28(1-14 Tyr) abolished all specific staining; pre-absorption of the antibody with other peptides had no effect. Specificity details of the other antisera used have been given elsewhere^{16,17}. The antiserum raised to somatostatin-28(1-14 Tyr) gave much more intense staining of cell bodies, dendrites and fibres than the antiserum raised to somatostatin-14 (in agreement with the reports of Morrison *et al.*³⁸ for the rat brain). Therefore, the data reported here are obtained using the somatostatin-28(1-14 Tyr) antiserum.

analogous to those obtained in silver-impregnated sections (Fig. 2); these degenerate morphological profiles were present in hippocampal areas (subiculum, CA1 and hilus of dentate gyrus) and the amygdala (primarily medial and cortical nuclei). Conversely, somatostatin immunoreactivity in the basal ganglia (caudate nucleus and putamen) was unchanged, with normal degrees of fibre innervation and no evidence of neuronal degeneration. In silver-impregnated sections which were subsequently immunostained, neurones with argyrophilic tangles were often somatostatin-positive (Fig. 3). These silver/somatostatin-positive structures (SSPS) were observed in the hippocampus (Fig. 3*a, c*) cortical areas (layers II and III, less often in layers V and VI; Fig. 3*b*) and amygdala. In all cases, careful scanning revealed somatostatin-positive neurones which were not degenerate or impregnated with silver (sometimes in the middle of clusters of SSPS) and tangles unstained for somatostatin (Fig. 3*c*). No specific relationship was discernible between plaques and somatostatin-positive structures. Occasionally some plaques seemed to be associated closely with somatostatin-positive fibres.

To establish the specificity of the relationship between somatostatin-positive cells and tangles, additional sections were processed for immunocytochemistry or for silver impregnation followed by immunocytochemistry using specific antibodies to cholecystokinin-octapeptide, vasoactive intestinal polypeptide¹⁶ and neuropeptide Y¹⁷ (all present in intrinsic cortical neurones). Fibres containing these peptides were associated with plaques. None of these peptides was decreased in Alzheimer's disease, co-localized with neuronal tangles or displayed appreciable

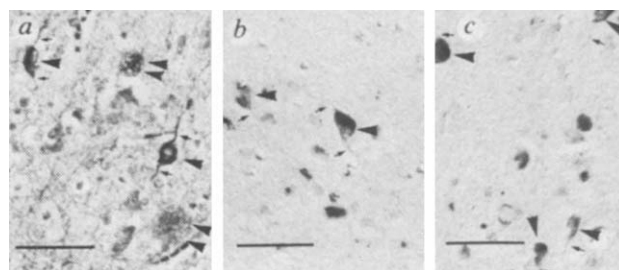


Fig. 2 *a*, Silver-impregnated section of temporal cortex showing typical tangles (single arrowhead) and plaques (double arrowheads) in a case of Alzheimer's disease. Note how dendrites of tangles are thickened and degenerate. *b*, Degenerate neuronal profiles of somatostatin-28-immunoreactive neurones in temporal cortex (layer III) of the Alzheimer's case (arrows). Note degenerating dendrites with almost total absence of staining (small arrows). *c*, Degenerate somatostatin-28-positive neurones in subiculum of hippocampus (arrows). Note presence of degenerating dendrites (small arrows). Scale bars, 50 μ m.

numbers of conspicuously abnormal morphological profiles (Fig. 3*d*), with one partial exception. In the most severely affected cases (judged by neuropathological criteria), some neuropeptide Y-containing neurones in deeper cortical layers were degenerate and co-localized with neuronal tangles. In some cortical and subcortical neurones, neuropeptide Y is colocalized with somatostatin^{18,19}. However, neuropeptide Y content is largely unchanged in Alzheimer's disease²³; thus, the observed degeneration may be a late change. These results agree with radioimmunoassay studies demonstrating (1) that somatostatin concentrations are higher in the temporal than other cortices and (2) that although somatostatin is decreased in Alzheimer's disease, other peptides (including neuropeptide Y) are relatively unaffected^{11,13,14,20}.

Both plaques and tangles are related quantitatively to dementia scores²¹⁻²³; both indices of pathology are also associated with the decreases in choline acetyltransferase (CAT) in the frontal and temporal cortex²¹⁻²⁴. Indeed, reports of CAT and acetylcholinesterase staining in plaques has led to the proposal that plaques are composed of (at least in part) degenerating cholinergic terminals²⁵. The relationship between plaques and CAT has prompted studies on the basal nucleus of Meynert—the source of almost all the cortical cholinergic innervation. This nucleus shows considerable cell loss and/or atrophy in Alzheimer's disease, as do the cell bodies of origin of the monoaminergic projections to the cerebral cortex, for example, the locus coeruleus²⁶⁻²⁸. These findings have prompted speculations that Alzheimer's is a disease of the isodendritic core²⁹, the primary lesion being subcortical (and cholinergic) and the cortical pathology resulting from transsynaptic degeneration caused by the loss of ascending fibres. Therefore, the plaques and tangles are viewed "as a secondary response to the loss of ascending inputs"²⁹. If this were correct, a loss of neurones in the basal nucleus of Meynert would lead to a drop in cortical CAT and somatostatin. In rats, lesions to the basal nucleus of Meynert do lead to cholinergic deficits but cortical somatostatin levels remain unchanged³⁰. Conversely, lesions of the cortex (which produce somatostatin losses along with losses of other intrinsic transmitters in both rats and man) result in retrograde degeneration of cholinergic cells in the basal nucleus of Meynert similar to that seen in Alzheimer's disease^{31,32}. These reports and the correlations between cortical pathology and symptoms imply that the cortex is the seat of pathology and that, in particular, the temporal cortex is the main focus of the disease process. The findings reported here that some plaques (which may include degenerating cholinergic terminals) contain somatostatin fibres (confirming a previous observation)³³ and reports that plaques also contain dendrites and axons of diverse local neurones and perhaps glial elements³⁴ indicate that plaques may be a neurochemically nonspecific consequence of the disease process.

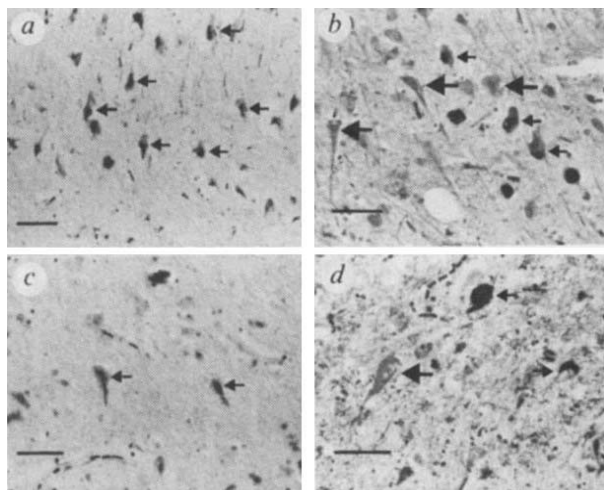


Fig. 3 Silver-stained sections (in which tangles stain black) were immunostained subsequently to detect the presence of somatostatin immunoreactivity (dark precipitate). In these sections many silver/somatostatin-positive structures (SSPS, dark staining) are seen in temporal regions in Alzheimer's cases. *a*, The subiculum of the hippocampus showed numerous SSPS (arrowed) as did *b*, neurones (arrowed) in layers II, III and V of the parahippocampal gyrus. *c*, The CA1 pyramidal region of the hippocampus also contained many SSPS (small arrows); it was observed often that even in regions which showed numerous SSPS, apparently normal somatostatin-28-positive neurones could still be observed (large arrows). *d*, Staining with antiserum to other peptides, for example vasoactive intestinal peptide, demonstrated that in the cortex, tangles (small arrows) did not occur in vasoactive intestinal peptide-immunoreactive neurones (large arrow). Scale bars, 50 μ m.

The findings demonstrate a strong link between the formation of neuronal tangles (their presence highly correlated with the symptoms of dementia) and the decrease of immunoreactivity to somatostatin (the only intrinsic cortical transmitter known to be affected) in the temporal cortex (pathologically the area most affected). We suggest that somatostatin neurones in the temporal cortex are a primary focus for the disease process and that the loss of somatostatin content is an early event in the production of symptoms and the subsequent (or concomitant) cortical atrophy. The cholinergic deficit and atrophy of the basal nucleus of Meynert may be a secondary consequence of the loss of cortical somatostatin neurones from a region which provides the major input to the basal nucleus³⁵. The reason for the selectivity of the disease process is unknown but presumably is related to the physiological properties of susceptible somatostatin neurones in the cortex. The structure of somatostatin itself (particularly the helical fibril-forming noncyclic form³⁶) or the recently described somatostatin gene³⁷ may be relevant.

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A functional correlate for the dihydropyridine binding site in rat brain

D. N. Middlemiss & M. Spedding

Merrell Dow Research Institute, Strasbourg Centre, 16 rue d'Ankara, 67084 Strasbourg Cedex, France

Calcium channels, controlling the influx of extracellular Ca^{2+} and hence neurotransmitter release, exist in the brain¹. However, drugs classed as calcium antagonists and which inhibit Ca^{2+} entry through voltage-activated Ca^{2+} channels in heart and smooth muscle², seem not to affect any aspect of neuronal function in the brain at pharmacologically relevant concentrations³⁻⁶. Yet the dihydropyridine calcium antagonists (for example, nitrendipine) bind stereospecifically with high affinity to a recognition site on brain-cell membranes thought to represent the Ca^{2+} channel^{4,7,8} and consequently, the physiological relevance of these sites has been questioned⁹. However, activation of voltage-dependent Ca^{2+} channels can increase cytoplasmic Ca^{2+} and neurotransmitter release in neuronal tissue¹⁰. We show here that Bay K 8644, a dihydropyridine Ca^{2+} -channel activator^{11,12}, can augment K^{+} -stimulated release of serotonin from rat frontal cortex slices and that these effects can be antagonized by low concentrations of calcium antagonists. As ^3H -dihydropyridine binding to cortical membrane preparations resembles the binding in heart and smooth muscle where there are good functional correlates^{8,13} we conclude that the dihydropyridine binding sites in the brain represent functional Ca^{2+} channels that can be unmasked under certain circumstances.

We have used K^{+} -induced depolarization of rat cortical slices to stimulate Ca^{2+} entry and evoke ^3H -serotonin release from the cortical serotonergic neurones as a functional correlate of the dihydropyridine binding site in brain tissue. Slices of the rat frontal cortex were incubated in Krebs medium with ^3H -serotonin to label specifically the neurotransmitter stores in serotonergic nerve terminals. The slices were then superfused with physiological medium and exposed to two stimuli of Krebs medium containing elevated K^{+} (25 mM). The first stimulus (S_1) occurred 38-42 min after the start of the superfusion and the second (S_2) at 78-82 min. ^3H -serotonin release is coupled closely to Ca^{2+} entry¹⁴ and K^{+} -evoked release in our experiments was entirely Ca^{2+} dependent. Drugs (when present) were added at $t = 58$ min and their effects quantified by the standard method of comparing the efflux of radioactivity evoked by the first K^{+} stimulus with that evoked by the second K^{+} stimulus (S_2/S_1 ratio). Effects on basal release rates were quantified by measuring the ratio of basal outflow immediately before S_1 compared with that immediately before S_2 .

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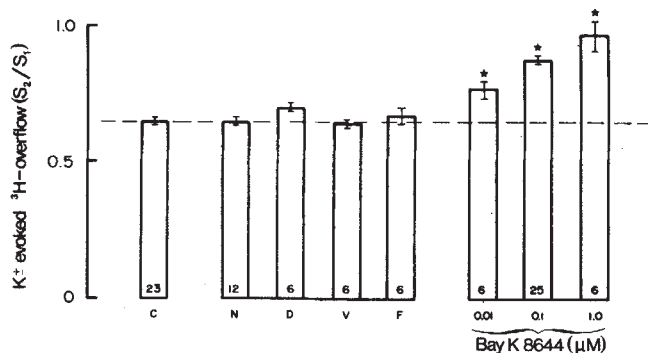


Fig. 1 Effect of calcium antagonists and Bay K 8644 on K^+ -evoked release of 3H -serotonin from rat frontal cortex slices. C, control; N, nifedipine (1 μM); D, diltiazem (1 μM); V, verapamil (1 μM); F, flunarizine (1 μM).

Methods. The frontal cortices from two male Sprague-Dawley rats (200–250 g) were dissected rapidly and chopped in two directions at 250 μm on a McIlwain brain-tissue chopper. The slices (total tissue weight 200–250 mg) were incubated for 15 min at 37 $^{\circ}C$ in 5 ml Krebs/Ringer-bicarbonate solution (composition (mM): NaCl 135, KCl 5, $NaHCO_3$ 25, $MgSO_4$ 1, KH_2PO_4 1.25, $CaCl_2$ 2, glucose 10; gassed with 95% O_2 and 5% CO_2) containing 0.1 μM 3H -serotonin (specific activity 28.3 Ci $mmol^{-1}$) and 10 μM pargyline. After three washes with 5 ml Krebs buffer, 50- μl aliquots of the slices (~20 mg tissue) were transferred to each chamber (100 μl vol) of a superfusion apparatus and superfused at a rate of 0.38 ml min^{-1} with Krebs buffer containing 3.2 μM paroxetine to prevent the re-uptake of serotonin into the nerve endings. After 30 min superfusion ($t = 30$ min), 18 successive 4-min fractions were collected ($t = 30$ to $t = 102$ min). The slices were exposed to two separate 4-min stimuli by Krebs buffer containing 25 mM K^+ ions (isosmolar replacement of NaCl by KCl) starting at $t = 38$ and 78 min. Drugs were added to the superfusate at $t = 58$ min and were present throughout the rest of the superfusion period. After the radioactivity in the fractions and that remaining in the tissue slices had been determined by liquid scintillation counting, the fractional efflux rate of each fraction was determined. The magnitude of the efflux evoked by the first stimulus (S_1) was then compared with that evoked by the second stimulus (S_2) by measuring the additional efflux evoked by the 25 mM K^+ Krebs during the time period $t = 38$ –58 min (S_1) and 78–98 min (S_2). For the purpose of this calculation, a linear decline in the efflux of 3H was assumed between $t = 38$ –62 min and $t = 78$ –102 min for the calculation of S_1 and S_2 respectively. At $t = 42$ min, control basal release rates in the absence of added drugs were $0.71 \pm 0.02\%$ tissue stores per min (mean $\pm$ s.e.m., $n = 29$). The additional release evoked by the first 4-min exposure to 25 mM K^+ Krebs (S_1) was $10.20 \pm 0.48\%$ tissue stores ($n = 29$) and to the second exposure (S_2) $6.72 \pm 0.36\%$ tissue stores ($n = 29$). Drug effects were quantified by calculating the S_2/S_1 ratio. Results are mean $\pm$ s.e.m. Numbers in the histograms indicate the number of separate experiments. Statistical significance of drug effects compared with controls was assessed using a two-tailed Mann-Whitney U test (* $P < 0.05$).

Although the K^+ -evoked 3H -serotonin release was entirely Ca^{2+} dependent, a range of calcium antagonists with disparate structures and different sites of action did not affect 3H -serotonin release at concentrations which cause considerable antagonism at Ca^{2+} channels in smooth muscle preparations¹⁵ (Fig. 1). This lack of effect on K^+ -evoked release was accompanied by a small but non-significant enhancement of basal 3H release, consistent with the negligible effects of calcium antagonists on neurotransmitter release reported previously^{5,6}.

Bay K 8644 both activates Ca^{2+} channels^{11,12} and increases rather than antagonizes Ca^{2+} entry¹⁶. It binds to the dihydropyridine recognition site and, in the rat cortex, displaces 3H -nimodipine binding with a K_i of 30 nM (ref. 17). Basal release of 3H -serotonin from rat frontal cortex slices was not modified significantly by Bay K 8644 (0.01–1 μM), but K^+ -evoked serotonin release was augmented in a concentration-dependent manner (0.01–1 μM ; Fig. 1). Thus, in the presence of 1 μM Bay 8644, the S_2/S_1 ratio was 0.97 ± 0.06 ($n = 6$), an

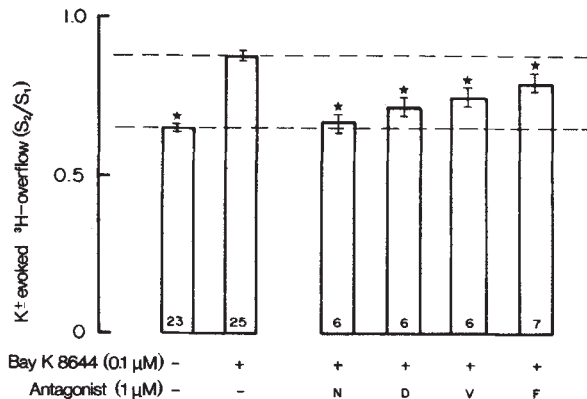


Fig. 2 Effect of calcium antagonists on the augmentation of K^+ -evoked release of 3H -serotonin from rat frontal cortex slices by Bay K 8644. N, nifedipine; D, diltiazem; V, verapamil; F, flunarizine. Experiments were performed as described in Fig. 1 legend. Bay K 8644 (0.1 μM) and the relevant antagonist (1 μM) were added to the superfusion medium at $t = 58$ min and were present throughout the rest of the experiment. Results are mean $\pm$ s.e.m. Numbers in the histograms indicate the number of separate experiments. Statistical significance of effects, compared with Bay K 8644 alone, were assessed using a two-tailed Mann-Whitney U test (* $P < 0.05$).

increase of 49% above the control value ($S_2/S_1 = 0.65 \pm 0.01$; $n = 23$). In this respect the effects of Bay K 8644 on 3H -serotonin release resemble the effects on K^+ -induced contractions in smooth muscle, where the compound increases tone only when the Ca^{2+} channels are stimulated by K^+ -depolarization¹². Furthermore, the concentrations of Bay K 8644 required to augment 3H -serotonin release were similar to those required to displace 3H -nimodipine binding in this tissue¹⁷. A submaximal concentration of Bay K 8644 (0.1 μM), which increased the S_2/S_1 ratio by 35% (S_2/S_1 ratio in the presence of 0.1 μM Bay K 8644 = 0.88 ± 0.02 ; $n = 25$), was chosen to investigate antagonist interactions.

The concentrations of the calcium antagonists which had no effect on K^+ -evoked 3H -serotonin release attenuated the stimulant effects of Bay K 8644 (Fig. 2). These concentrations have been shown to block Ca^{2+} -induced contractions in smooth muscle in the presence of Bay K 8644 (ref. 18); however, these concentrations are sufficiently low to exclude effects on ion channels other than the Ca^{2+} channel².

This interpretation is supported by experiments showing that the augmentation of K^+ -evoked release induced by the serotonin autoreceptor antagonist methiothepin was not attenuated by nifedipine. Thus, methiothepin (0.3 μM added after S_1) increased the S_2/S_1 ratio to 0.94 ± 0.05 (mean $\pm$ s.e.m.; $n = 6$) and a similar augmentation was also seen if nifedipine (1 μM) was added to the superfusate at the same time as the methiothepin (S_2/S_1 ratio 1.02 ± 0.06 ; not significant against methiothepin alone; $n = 6$). The stimulant effects of Bay K 8644 can therefore be attributed to stimulation of voltage-dependent Ca^{2+} channels which, in the presence of this activator, resemble those in smooth muscle cells¹⁸ or in cells derived from the adrenal glands^{19–22} in their susceptibility to organic calcium antagonists. Previous studies using tissues derived from sources other than the brain give conflicting evidence about the effects of Bay K 8644 on transmitter release^{19,22,23}. The reasons for these differences are not yet understood, but may reside in the nature of the different stimuli used to release the transmitters. Thus, Bay K 8644 can augment K^+ -evoked release of catecholamines from chromaffin tissue^{19,22} but not noradrenaline release from sympathetic neurones evoked by supramaximal electrical stimulation²³. We have observed that Bay K 8644 (0.1 μM) failed to augment K^+ -evoked release of 3H -serotonin from rat frontal cortex slices when the K^+ stimulus was increased to 50 mM (data not shown). We therefore consider that Bay K 8644 will only augment neurotransmitter release in conditions of submaximal stimulation. Nevertheless, our experiments have shown clearly that

Ca^{2+} channels in the brain differ from those in the heart and smooth muscle because they become sensitive to organic calcium antagonists only in the presence of Bay K 8644. One explanation for these apparent differences between central and peripheral Ca^{2+} channels may result from the recent discovery that Ca^{2+} channels comprise several subunits²⁴⁻²⁶ differentially affected by organic calcium antagonists²⁷. It is therefore possible that the subunits of the Ca^{2+} channel in nervous tissue are arranged differently from those in smooth muscle and the presence of Bay K 8644 is required to induce a conformational change, not only activating the channels but also conferring sensitivity to the antagonists. Such an effect could have important consequences if neuronal Ca^{2+} channels could be activated similarly in physiological or pathological conditions. Thus, calcium antagonists would have little effect on normal neuronal function but

may cause marked inhibition of excess Ca^{2+} entry following certain stimuli. Such stimuli may occur in pathological conditions as the anoxia-induced noradrenaline release from nerves in the heart is susceptible to calcium antagonists²⁸.

Thus, dihydropyridines have a defined functional role in the control of neurotransmitter release, affecting release at similar concentrations to those at which they bind to neuronal tissues¹⁷ and to those at which they affect smooth muscle cells¹⁸. The striking effects of Bay K 8644 in our experiments suggest that this Ca^{2+} channel activator will prove a useful tool with which to evaluate the functional effects of calcium antagonists in the brain.

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Identification of a distinct synaptic glutamate receptor on horizontal cells in mudpuppy retina

Malcolm M. Slaughter

Department of Biophysical Sciences, State University of New York, Buffalo, New York 14214, USA

Robert F. Miller

Departments of Ophthalmology, Physiology and Biophysics, Washington University, St Louis, Missouri 63110, USA

The separation of ON and OFF channels and the development of an antagonistic surround occur at the first synapse in the vertebrate retina¹⁻³. This functional differentiation is mediated by the action of the photoreceptor neurotransmitter on the ON bipolar, OFF bipolar and horizontal cells, respectively. Glutamate mimics the action of the photoreceptor transmitter on all second-order neurones in fish^{4,5}, amphibian⁶ and mammalian⁷ retinas. The diversity of cellular responses produced by one neurotransmitter raises the possibility of multiple postsynaptic receptor-ionophore complexes. We reported previously that one glutamate analogue, 2-amino-4-phosphonobutyrate, reveals that the ON bipolar synaptic receptor is pharmacologically different from those of other second-order neurones^{8,9}. The results presented here demonstrate that another glutamate analogue, D-O-phosphoserine, selectively antagonizes the synaptic responses of horizontal cells. Taken together, these findings indicate that there are three glutamate-like receptor subtypes in the outer retina and suggest a correlation between receptor subtype and the physiological properties of second-order neurones.

Experiments were performed on the superfused retina-eyecup preparation of the mudpuppy, *Necturus maculosus*. The hemisected eyecup was superfused with amphibian Ringer's solution (111 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 , 1 mM MgCl_2 , 10 mM dextrose, 5 mM HEPES, pH 7.80) to which pharmaco-

logical agents could be added. Standard intracellular electrophysiological techniques were used to evaluate the effects of drugs on the light-driven synaptic responses of second-order neurones. A complete description of the methodology has been published previously^{6,10}.

Glutamate analogues such as kainic acid and quisqualic acid mimic the action of the photoreceptor neurotransmitter on all mudpuppy second-order neurones⁶. In the present experiments, we examined the effects of the D form of a glutamate analogue, O-phosphoserine, on exogenously applied glutamate analogues and on the endogenous photoreceptor neurotransmitter. When the retina was superfused with 5 mM D-O-phosphoserine, photoreceptor light responses were unchanged or only slightly enhanced. However, the drug had a selective effect on synaptic transmission between photoreceptors and second-order neurones. The photoreceptor neurotransmitter is released maximally in the dark, causing horizontal cells to be depolarized¹¹⁻¹³. Figure 1a shows that D-O-phosphoserine at 2.5 mM hyperpolarized the 'dark' membrane potential of the horizontal cell and diminished the light response by 50%, consistent with a blocking of the photoreceptor neurotransmitter. Both small spot- and annulus-evoked responses were similarly affected, and the antagonism was dose-dependent over the range 1-10 mM. In contrast to this action of D-O-phosphoserine on horizontal cells, the direct synaptic communication between photoreceptors and bipolar cells was not diminished by the drug. Figure 1b shows that the 'centre' response of OFF bipolars to small-spot or annular stimulation remained essentially unaltered during a relatively prolonged application of 5 mM D-O-phosphoserine. However, the antagonistic surround response was blocked (see Fig. 1 legend). The surround response of bipolar cells is believed to result from horizontal cell feedback¹⁴⁻¹⁹. Therefore, the maintenance of the centre response during the loss of the surround response in the OFF bipolar is consistent with a selective blocking of the horizontal cells. In Fig. 1c, D-O-phosphoserine was applied to the retina while recording from an ON bipolar cell; as with the OFF bipolar, the drug did not reduce the centre response but the antagonistic surround response was suppressed.

To explore the pharmacology of D-O-phosphoserine, we compared its action with that of known excitatory amino-acid agon-

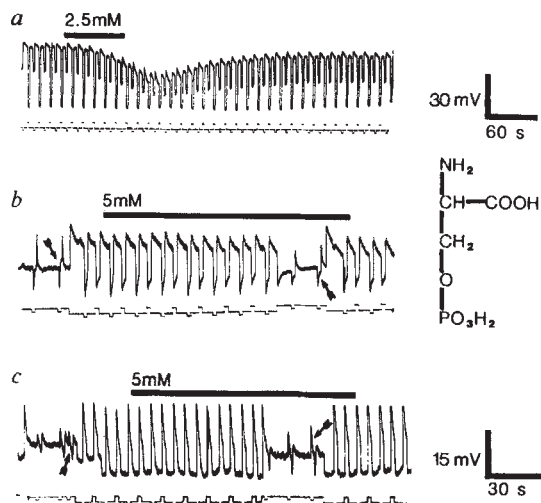


Fig. 1 Action of D-O-phosphoserine on the light responses of second-order neurones. *a*, 2.5 mM D-O-phosphoserine hyperpolarized and suppressed 50% of the synaptic responses of horizontal cells, while more prolonged application of twice this concentration did not diminish the centre responses of either OFF (*b*) or ON (*c*) bipolar cells. However, the sustained antagonistic surround response in bipolar cells, evoked by flashing an annulus during maintained small-spot illumination, was suppressed; this is shown by the reversal in polarity of the surround responses indicated by arrows before and during drug application. Bars above the traces indicate the duration of drug application; square pulses below the voltage traces mark the time of light stimuli. Upward pulses represent small spots (200 μ m) and downward pulses mark annular stimulation (internal diameter 400 μ m, external diameter greater than retinal diameter). Illumination, 5×10^{-9} W cm $^{-2}$. The lower calibration applies to *b* and *c*. The molecular formula for D-O-phosphoserine is shown at the right.

ists and antagonists. One antagonist that is effective on second-order neurones is *cis*-2,3-piperidine dicarboxylic acid (PDA), which blocks photoreceptor input to both OFF bipolars and horizontal cells¹⁹. At equivalent concentrations, D-O-phosphoserine and *cis*-2,3-piperidine dicarboxylic acid had a similar effect on the synaptic responses of horizontal cells (Fig. 2*a*) although PDA was a slightly more potent antagonist.

The photoreceptor-horizontal cell synapse is mediated by a glutamate-preferring receptor and kainic acid mimics the action of the photoreceptor neurotransmitter⁶. Therefore, we evaluated the ability of D-O-phosphoserine to antagonize the effects of exogenously applied kainic acid. Figure 2*b* illustrates that a brief application of 50 μ M kainic acid drives a horizontal cell towards the dark resting membrane potential and suppresses the light response, mimicking the endogenous transmitter. Figure 2*c* shows the same cell after recovery from the kainic acid-induced depolarization. D-O-phosphoserine at 3 mM produced a hyperpolarization and a reduction of the light response; in the continued presence of the drug, a more prolonged application of kainic acid had little effect on the neurone. γ -Aminobutyric acid (GABA) at 5 mM directly depolarizes mudpuppy horizontal cells to close to their dark membrane potential (M.S., unpublished observation); D-O-phosphoserine does not block this GABA-induced depolarization, indicating that the former is not acting by shunting horizontal cells towards an equilibrium potential below the dark membrane potential. These results suggest that D-O-phosphoserine suppresses the response of horizontal cells to light by blocking glutamate-like synaptic receptors.

Although it is not very potent, D-O-phosphoserine seems to possess a selectivity that may be useful as a pharmacological probe to identify and characterize excitatory amino-acid receptors. This ligand has also been reported to displace glutamate at some binding sites in rat forebrain²⁰. The action of D-O-

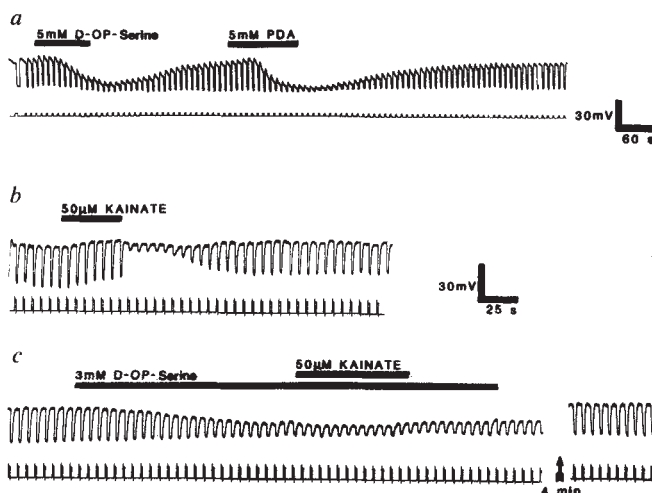


Fig. 2 *a*, Similarity of action of D-O-phosphoserine (D-OP-Serine) and *cis*-2,3-piperidine dicarboxylic acid (PDA; Cambridge Research Biochemicals), an excitatory amino-acid antagonist, on the light responses of a horizontal cell. The traces in *b* and *c*, from the same horizontal cell, show that D-O-phosphoserine suppresses the action of both kainic acid and the photoreceptor neurotransmitter. Diffuse light stimuli were used.

phosphoserine in the distal retina reveals a discrete glutamate receptor subtype at the horizontal cell synapse. We have reported previously that the ON bipolar cell possesses a synaptic glutamate receptor not found in other retinal neurones⁹. From these observations we conclude that there are three glutamate receptor subtypes in the outer plexiform layer and that each pharmacological subtype is found on a distinct physiological class of second-order neurone.

The reason for this receptor diversity and functional segregation is unclear; it may be related to receptor-ionophore coupling as it has been proposed that the synaptic potential of each class of second-order neurone is generated by a discrete ionic mechanism²¹⁻²³. Another possible explanation, suggested by noise analysis in dogfish and turtle retinas, is that the kinetics of synaptic communication differs among the second-order neurones²⁴⁻²⁶. This may be an important coding mechanism for information transfer mediated by postsynaptic receptors. The differentiation of excitatory amino-acid receptors in the distal retina may elucidate further the role of receptors in synaptic communication.

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Expression of interleukin-2 receptors as a differentiation marker on intrathymic stem cells

Rhodri Ceredig*, John W. Lowenthal*,
Markus Nabholz† & H. Robson MacDonald*

* Ludwig Institute for Cancer Research, Lausanne Branch, and
† Genetics Unit, Swiss Institute for Experimental Cancer Research,
1066 Epalinges, Switzerland

The thymus is regarded as the primary site for T-cell lymphopoiesis¹, but very little is known about the lineage interrelationships of cells within that organ. At least four subpopulations of mouse thymocytes can be defined on the basis of staining with monoclonal antibodies directed against the T-cell differentiation antigens Lyt-2 and L3T4 (ref. 2). Thus immunocompetent (medullary) thymocytes, like peripheral T cells, express either Lyt-2 (cytotoxic phenotype) or L3T4 (helper phenotype) but not both, whereas non-functional (cortical) thymocytes express both markers. In addition, a small subpopulation comprising 2–3% of cells in the thymus and expressing neither Lyt-2 nor L3T4 has recently been described². The latter cells have the properties of intrathymic 'stem cells' in that they are the first to appear in the embryonic thymus^{2,3} and at least some can be shown to give rise, both *in vivo* (ref. 4. and our unpublished data) and *in vitro*⁵, to other thymocyte subpopulations. We show here that 50% of Lyt-2⁺/L3T4⁺ cells in the adult thymus express receptors for the polypeptide growth hormone interleukin-2 (IL-2)⁶ whereas other cells in the thymus do not. Furthermore, immunohistochemical localization studies on frozen sections indicate a disperse distribution of IL-2 receptor-positive cells in both the cortex and medulla. These novel findings have potential implications in the context of current models of differentiation pathways within the thymus.

Very few positive cells (~2%) were observed when unfractionated thymocytes were exposed to anti-IL-2-receptor monoclonal antibody (PC61) and analysed by flow microfluorometry (FMF) (Fig. 1), in line with a previous report⁷. However, when the Lyt-2⁺/L3T4⁺ subpopulation was isolated by negative selection with a mixture of monoclonal antibodies and complement, clear staining of 51 ± 3% (mean ± s.d. of three experiments) of the cells was observed (Fig. 1). As Lyt-2⁺/L3T4⁺ cells account for 3% of total thymocytes², these data suggest (but do not

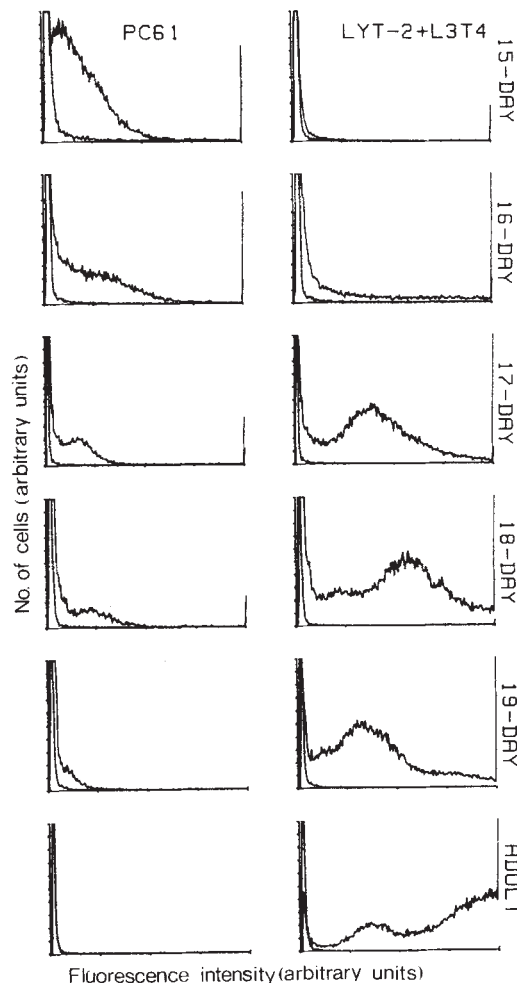


Fig. 2 Ontogeny of IL-2 receptor expression in the developing embryonic thymus. C57BL/6 Thymocytes of the indicated gestational ages, and control adult thymocytes, were stained with PC61 monoclonal antibody (left panels) and analysed by FMF as described in Fig. 1 legend. For comparison, the proportion of Lyt-2⁺/L3T4⁺ cells in the same population was determined by simultaneous staining with anti-Lyt-2 and anti-L3T4 monoclonal antibodies² (right panels). The proportions of PC61⁺ and Lyt-2⁺/L3T4⁺ cells, respectively, were: day 15 (66%, 88%), day 16 (40%, 60%), day 17 (15%, 30%), day 18 (5%, 15%), day 19 (4%, 10%), adult (2%, 3%).

Fig. 1 Flow microfluorimetric (FMF) analysis of IL-2 receptor expression by Lyt-2⁺/L3T4⁺ thymocytes (lower row). Upper row, normal thymus controls. Linear fluorescence or forward light scatter (FLS) histograms are expressed in arbitrary units. Dotted lines represent staining with the fluorescent conjugate only.

Methods. C57BL/6 thymocytes ($50 \times 10^6 \text{ ml}^{-1}$) were incubated with a mixture of rat IgM monoclonal antibodies directed against Lyt-2 (3.168.8.1²¹, provided by F.W. Fitch) and L3T4 (LICR.LAU.RL172.4) at a 1:10 final dilution of hybridoma culture supernatant. The latter monoclonal antibody was obtained by fusing spleen cells from a rat immunized with the L3T4-positive thymoma EL-4 to the non-secreting mouse myeloma X63-Ag8.653 and screening the hybrids for selective complement-dependent lysis of L3T4-positive thymomas (R.C., R. K. Lees and H. R. MacD., unpublished data). After incubation for 15 min at 37 °C, rabbit complement was added to a 1:20 final dilution for an additional 45 min. Treated cells were passed over Ficoll-Hypaque (specific gravity 1.077) and washed twice. Viability was >90%, and cell recovery was $1.0 \pm 0.3\%$ (mean ± s.d. of six experiments). For FMF analysis, aliquots of 1×10^6 control or Lyt-2⁺/L3T4⁺ thymocytes in 100 µl were incubated at 4 °C with appropriate (saturating) concentrations of rat monoclonal antibodies directed against either Lyt-2 (53-6.7²², provided by J. Ledbetter), L3T4 (H129-19²³, provided by M. Pierres) or the murine IL-2 receptor (PC61). After 30 min, 50 µg of fluorescein isothiocyanate-conjugated rabbit anti-rat immunoglobulin (Nordic Laboratories) was added for an additional 30 min at 4 °C. Control samples were stained with the second step reagent alone. All samples were then passed on a flow cytometer (FACS II, Becton and Dickinson) gated to exclude nonviable cells²⁴.

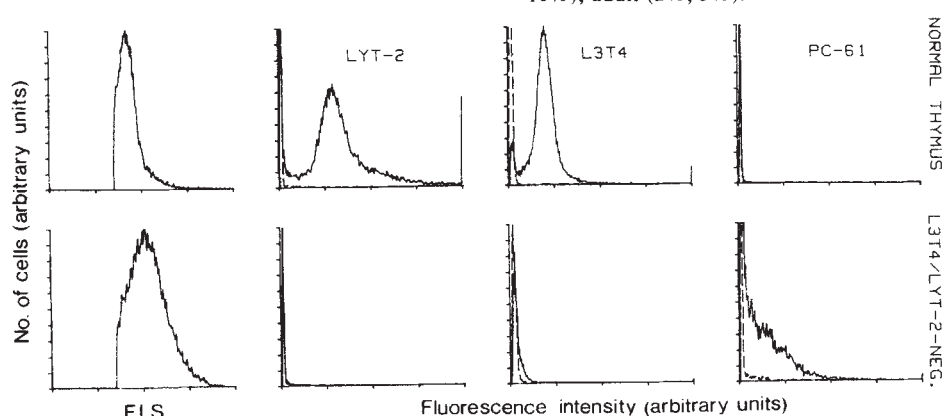
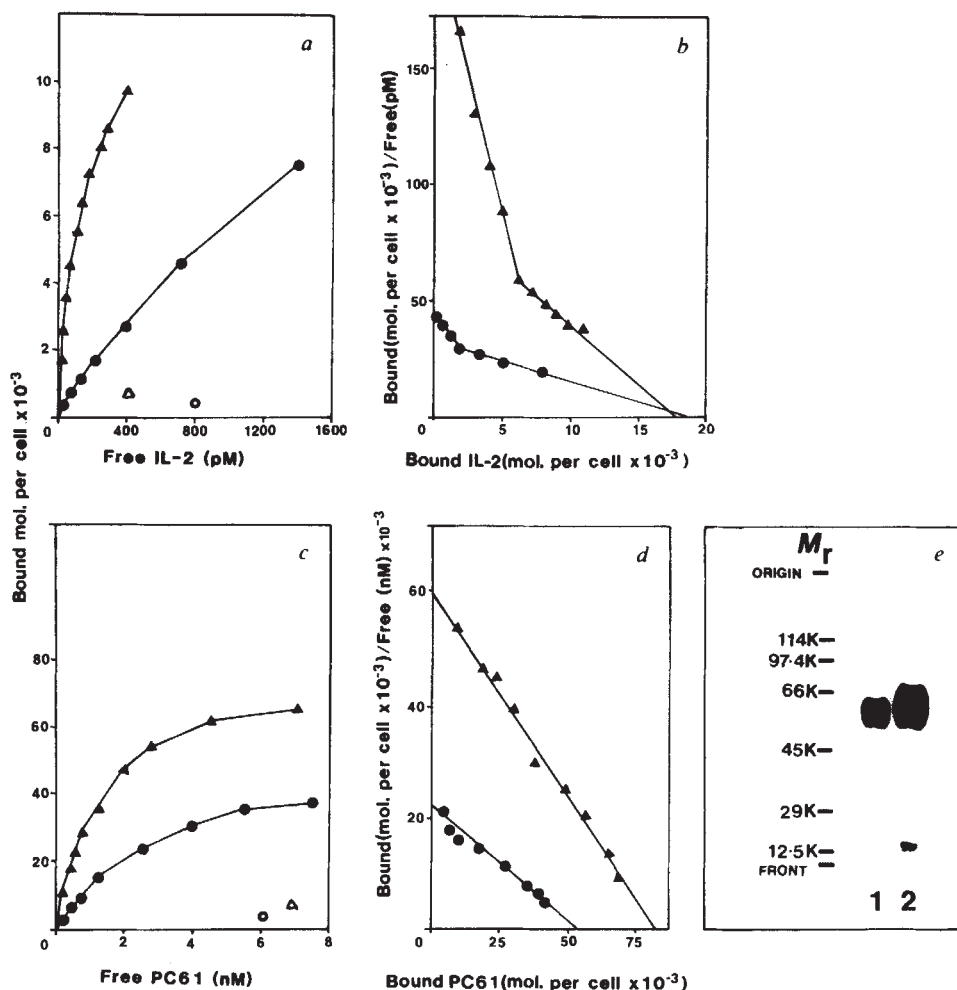


Fig. 3 Equilibrium binding and immunoprecipitation analysis of IL-2 receptors on $\text{Lyt-2}^-/\text{L3T4}^-$ thymocytes and Con A blasts. $\text{Lyt-2}^-/\text{L3T4}^-$ thymocytes (prepared as described in Fig. 1) and Con A blasts (prepared by culturing 2×10^6 C57BL/6 spleen cells for 4 days together with $1.5 \mu\text{g ml}^{-1}$ Con A and 10% (v/v) supernatant from phorbol myristate acetate-stimulated EL-4 cells) were passed over Ficol-Hypaque and washed extensively. ^3H -IL-2 from biosynthetically labelled Jurkat cell supernatant was purified by immunoaffinity chromatography on a column of Aphigel coupled to DMS-3 anti-IL-2 monoclonal antibody (provided by K. A. Smith) as described in detail elsewhere²⁵. ^3H -PC61 was purified from biosynthetically labelled hybridoma culture supernatant by dialysis followed by passage over protein A-Sepharose. For the binding assays (a-d) aliquots of $1-3 \times 10^6$ cells in $200 \mu\text{l}$ were incubated for 20 min at 4°C with varying concentrations of ^3H -IL-2 or ^3H -PC61. Free and cell bound radioactivity was measured by liquid scintillation counting following centrifugation of samples through a paraffin/silicon oil gradient¹⁰. The number of bound IL-2 and PC61 molecules per cell was calculated given the specific activities of $2,500 \text{ c.p.m. ng}^{-1}$ and $210 \text{ c.p.m. ng}^{-1}$, respectively. The M_r of IL-2 and PC61, as judged by SDS-PAGE, is 15,000 (15K) and 170K, respectively. Nonspecific binding was measured by incubating the cells in the presence of a 100-fold excess of the relevant unlabelled ligand. Equilibrium binding of IL-2 (Δ), Con A blasts: $\bullet$, thymocytes) is shown in a after subtraction of non-saturable binding (open symbols). The open symbols represent ^3H -IL2 binding in the presence of either excess cold IL-2 or PC61 mAb. For Con A blasts, binding was saturable at a free IL-2 concentration of $\sim 2 \text{ nM}$, whereas the binding to $\text{Lyt-2}^-/\text{L3T4}^-$ thymocytes was not saturable at this concentration. Equilibrium binding of ^3H -PC61 is shown in c (same symbols as a). Scatchard plots of these data are shown in b and d. The number of high- and low-affinity IL-2 binding sites was estimated by computer analysis of the two linear components of the Scatchard plots. For immunoprecipitation analysis (e), $\text{Lyt-2}^-/\text{L3T4}^-$ thymocytes (3×10^6 cells, lane 1) or Con A blasts (10×10^6 cells, lane 2) were labelled by the enzyme catalysed surface iodination technique of Hubbard and Cohn²⁶ modified as described previously²⁷. The labelled cells were lysed in 0.5 ml of cold 1% recondensed Triton X-114 in 50 mM Tris-HCl ($\text{pH } 7.7$) containing 0.3 M NaCl and 5 mM EDTA. The aqueous and detergent phases were separated according to Bordier²⁸. The lysates were pre-cleared for immunoprecipitation by sequential addition of $5 \mu\text{l}$ normal rat serum, $20 \mu\text{l}$ rabbit anti-rat immunoglobulin and $12.5 \mu\text{l}$ Protein A-Sepharose 4B (twice). The pre-cleared lysates were then incubated for 60 min with purified PC61 antibody coupled to Sepharose 4B, followed by extensive washing in phosphate buffer ($\text{pH } 8.2$). The immunoprecipitates were analysed by SDS-PAGE according to Laemmli²⁹ on 7.5–15% gradient gels. Samples were reduced with 0.1 M dithiothreitol and denatured by boiling in 4% SDS. After electrophoresis, gels were autoradiographed at -70°C on Kodak XAR film. Exposure times were 8 days (lane 1) and 4 days (lane 2). M_r standards were β -galactosidase (114K), phosphorylase b (97.4K), bovine serum albumin (66K), ovalbumin (45K), carbonic anhydrase (29K) and cytochrome c (12.5K).



prove) that all PC61⁺ cells are contained within this subpopulation. No Lyt-2^+ or L3T4^+ cells were detected after the antibody treatment; however, $\sim 90\%$ of the cells expressed Thy-1. The fluorescence distribution of PC61⁺ cells was heterogeneous, with a mean intensity corresponding to 76% of that obtained for IL-2-dependent cells from a cytotoxic T-cell line⁸ (included in each experiment as a positive control). Forward light scatter (FLS) analysis of $\text{Lyt-2}^-/\text{L3T4}^-$ cells indicated that they were considerably larger than most thymocytes (124 ± 4 arbitrary units as compared with a value of 100 units for small cortical thymocytes). Simultaneous FLS and PC61 fluorescence analysis of the $\text{Lyt-2}^-/\text{L3T4}^-$ subpopulation did not reveal any size distinction between PC61⁺ and PC61⁻ cells.

The early (day 15) embryonic thymus, which is known from transplantation experiments to be a rich source of intrathymic stem cells⁹, is also enriched for $\text{Lyt-2}^-/\text{L3T4}^-$ cells², but their proportion decreases rapidly during subsequent development².

When thymocytes from embryos of differing gestational ages were stained with PC61 and analysed by FMF, a good correlation was observed between the proportion of PC61⁺ and $\text{Lyt-2}^-/\text{L3T4}^-$ cells (Fig. 2).

Several other independent experimental approaches were used to verify IL-2 receptor expression on $\text{Lyt-2}^-/\text{L3T4}^-$ thymocytes. Equilibrium binding assays using biosynthetically labelled immunoaffinity-purified IL-2 (^3H -IL-2) demonstrated that adult $\text{Lyt-2}^-/\text{L3T4}^-$ thymocytes bound IL-2, and that this binding could be competed for by excess unlabelled IL-2 or by purified PC61 antibody (Fig. 3a). This latter result further confirms the fact that PC61 reacts with the IL-2 receptor. Scatchard plot analysis of the IL-2 binding data (Fig. 3b) shows that $\text{Lyt-2}^-/\text{L3T4}^-$ thymocytes express two classes of receptors with differing affinity for IL-2 (apparent dissociation constant, K_D , of 96 and $8,130 \text{ pM}$). The total number of IL-2 binding sites per cell was about 14,700, with 1,200 receptors being of the high-

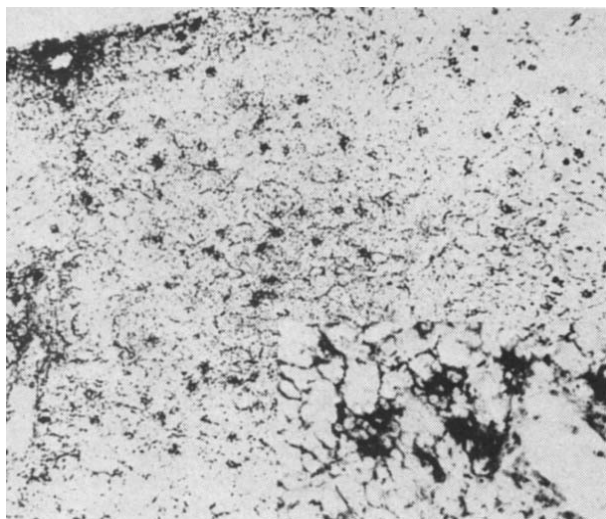


Fig. 4 *In situ* localization of PC61⁺ cells in the thymus. Thymuses from 6-week-old C57BL/6 mice were removed and snap frozen as previously described¹⁷. PC61 monoclonal antibody was directly coupled to biotin and the bound antibody revealed by adding an avidin-peroxidase conjugate³⁰ followed by freshly prepared 3,3-diaminobenzidine solution containing 0.06% H₂O₂. At low magnification ($\times 450$), positively stained cells are seen as individual cells and small clusters scattered throughout the cortex. Insert ($\times 890$): positive cells are also seen surrounding small blood vessel (bottom right) in medulla.

affinity class. By comparison, concanavalin A (Con A)-activated T lymphocytes expressed slightly more IL-2 receptors (37,000 total and 3,000 high-affinity binding sites) and bound ³H-IL-2 with higher affinity ($K_D = 19$ and 1,360 pM for high- and low-affinity receptors, respectively). Previous reports indicated that activated T lymphocytes expressed only a single class of high-affinity IL-2 receptors¹⁰; however, it has recently become clear that activated T lymphocytes express two classes of receptors when very high concentrations of ³H-IL-2 are used¹¹.

The significance of the reduced affinity of IL-2 receptors on adult *Lyt-2*⁻/*L3T4*⁻ thymocytes compared with Con A blasts is not known. For antigen- or mitogen-activated mature T cells and IL-2-dependent T-cell lines, there is a good quantitative correlation between the K_D of high-affinity IL-2 receptors (~ 20 pM) and the IL-2 concentration required for half-maximal proliferation⁶ (by definition, 1 unit ml⁻¹). In the present case, saturation of IL-2 receptors on *Lyt-2*⁻/*L3T4*⁻ cells would be more difficult to achieve because of their low affinity ($K_D = 96$ pM), and indeed we and others (see accompanying paper¹²) have thus far observed only marginal proliferation (as judged by ³H-thymidine incorporation) when such cells are cultured in the presence of extremely high concentrations (100–300 units ml⁻¹) of affinity-purified or recombinant IL-2.

Equilibrium binding with biosynthetically labelled purified PC61 monoclonal antibody (³H-PC61) confirmed and extended the fluorescence results. Figure 3c shows that ³H-PC61 binding to adult *Lyt-2*⁻/*L3T4*⁻ thymocytes and Con A blasts is saturable and can be competed for by excess unlabelled PC61 antibody. From Scatchard plots of these data (Fig. 3d), the total number of PC61 binding sites is 53,000 for thymocytes and 81,000 for Con A blasts, and both cell populations bind the monoclonal antibody with similar affinity (K_D of 2.1 nM and 1.3 nM, respectively). The reason for the apparent excess of PC61 over IL-2 binding sites is not clear, but a similar discrepancy has been observed for IL-2¹⁰ and anti-Tac¹³ binding to human HUT-102 cells.

Analysis by SDS-polyacrylamide gel electrophoresis (PAGE) under reducing conditions (Fig. 3e) illustrates that PC61 monoclonal antibody precipitates membrane components of similar apparent relative molecular mass ($M_r = 55,000$ –65,000) from

adult *Lyt-2*⁻/*L3T4*⁻ thymocytes and Con A blasts. These results are similar to those reported for a variety of human and mouse T-cell lines^{7,13}. In addition, bands of lower M_r (15,000–25,000) were precipitated from Con A blasts. The nature of this low- M_r material is unknown, but we find similar results with other T-cell lines and with an independently derived anti-IL-2-receptor monoclonal antibody (7D4⁷, kindly provided by T. R. Malek).

The apparently unique reactivity of PC61 antibody with *Lyt-2*⁻/*L3T4*⁻ thymocytes provides an opportunity to determine the anatomical location of these putative stem cells *in situ*. Immunohistochemical localization of PC61⁺ cells by the peroxidase method (Fig. 4) shows that individual cells (or small clusters) can be seen scattered more or less at random throughout both the thymic cortex and medulla. This distribution is at odds with the widely held view that thymic maturation proceeds sequentially from the subcapsular region through the cortex to the medulla¹⁴; however, it is consistent with early studies by Metcalf¹⁵ as well as the recent data of Ezine *et al.*¹⁶ and Ceredig and Schreyer¹⁷ suggesting a focal (clonal?) distribution of donor-derived (putative stem) cells repopulating the thymus in Thy-1 congenic radiation bone marrow chimaeras. It remains to be determined whether the foci of IL-2-receptor-bearing cells observed here and as recently reported by Takacs *et al.*³¹ are associated with a particular non-lymphoid component of the thymus.

The presence of IL-2 receptors on *Lyt-2*⁻/*L3T4*⁻ thymocytes raises interesting questions relevant to T-cell differentiation and activation. Among mature T cells, IL-2 receptor expression is always linked to an activation process dependent on antigen¹⁸ or mitogen¹⁹ binding. In this context, the apparently 'spontaneous' expression of IL-2 receptors on a subpopulation of intrathymic stem cells raises the possibility that receptor expression during the early stages of T-cell differentiation is not yet linked to specific antigenic stimulation. Alternatively, IL-2 receptor expression by these cells may reflect some transient 'antigen-like' differentiative stimulus (either cellular or humoral) intrinsic to the thymus. If the latter hypothesis is correct, the data presented here provide evidence that the T-cell specificity repertoire may result from *in situ* selection (either positive or negative) by self antigens within the thymus²⁰.

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Expression and function of interleukin-2 receptors on immature thymocytes

David H. Raulet

Center for Cancer Research and Department of Biology,
Massachusetts Institute of Technology, Cambridge,
Massachusetts 02139, USA

T-cell differentiation represents a unique system for studying mechanisms of lymphoid development because it occurs in a segregated site, the thymus, in which distinct subpopulations of thymocytes at various stages of differentiation can be defined on the basis of the differential expression of T-cell surface antigens as well as topography. There is particular interest in thymocyte differentiation because the genotype of radioresistant thymus cells influences the specificity repertoire of the pool of T cells that mature therein: that is, the major histocompatibility complex (MHC) antigens expressed by thymus cells bias the pool of maturing T cells towards recognition of antigens in the 'context' of the products of that MHC haplotype ('thymus education'; refs 1-3). Immature T cells with affinity for thymus MHC antigens are generally thought to undergo a stage of positive selection in the thymus⁴⁻⁶. Here we report that 30% of cells in the least mature adult thymocyte subpopulation yet defined, as well as 50% of immature fetal thymocytes, express receptors for interleukin-2 (IL-2, the T-cell growth factor) without *in vitro* induction, and will proliferate vigorously in an IL-2-dependent fashion if provided with co-stimulating mitogen.

Recent studies with monoclonal antibodies reactive with the T-cell antigens Lyt-2, L3T4 and Thy-1 have defined at least four subpopulations of thymocytes in the adult thymus. Thymocytes with the mature phenotypes L3T4⁺, Lyt-2⁻, Thy-1⁺ (helper T-cell phenotype) or L3T4⁻, Lyt-2⁺, Thy-1⁺ (cytolytic T-cell phenotype) appear late in fetal ontogeny^{7,8} and most are functional in *in vitro* assays of T-cell activities^{9,10}. These cells may include end-stage thymocytes awaiting export from the thymus although medullary thymocytes with these phenotypes may not be exported¹¹. A large subpopulation (~85%) of thymocytes are mostly small noncycling cells with the phenotype L3T4⁺, Lyt-2⁺, Thy-1⁺, a phenotype not yet found amongst peripheral T cells; the specific ontogenetic relationship of these cells to other thymocyte subpopulations (that is, whether they are intermediates) is presently obscure. A fourth thymus subpopulation (~3% of adult thymocytes) has the phenotype L3T4⁻, Lyt-2⁻, Thy-1⁺ and includes mostly blast cells, many of which are cycling¹². In addition to the immature phenotype of these cells, there is evidence that they represent the least mature thymocyte subpopulation yet defined: on transfer to irradiated recipients these cells, unlike other thymocytes, are able to repopulate the recipient thymus and give rise with time to each of the other thymocyte subpopulations¹². Furthermore, before day 17 of embryonic murine development most (80-95%) thymocytes have the L3T4⁻, Lyt-2⁻, Thy-1⁺ phenotype^{7,8}.

In our studies of the expression of IL-2 receptors by thymocytes, we used the recently described rat monoclonal antibodies 3C7 (IgG) and 7D4 (IgM) isolated by Malek and Shevach^{13,14}. These antibodies recognize different epitopes on the IL-2 receptor as they do not compete with each other for binding, and the binding of 3C7 (but not 7D4) is blocked by purified IL-2: both antibodies block IL-2-dependent proliferation and precipitate a similar polypeptide of relative molecular mass 50,000-60,000 (refs 13, 14).

Indirect immunofluorescent staining profiles of day 16 fetal thymocytes and adult lymph node cells with various monoclonal antibodies (Fig. 1) show that most fetal thymocytes (95%) are Lyt-2⁻, Thy-1⁺, whereas adult thymocytes are predominantly Lyt-2⁺, Thy-1⁺ cells. About 50% of fetal thymocytes bind the 3C7 anti-IL-2 receptor antibody and 30% bind the 7D4 antibody.

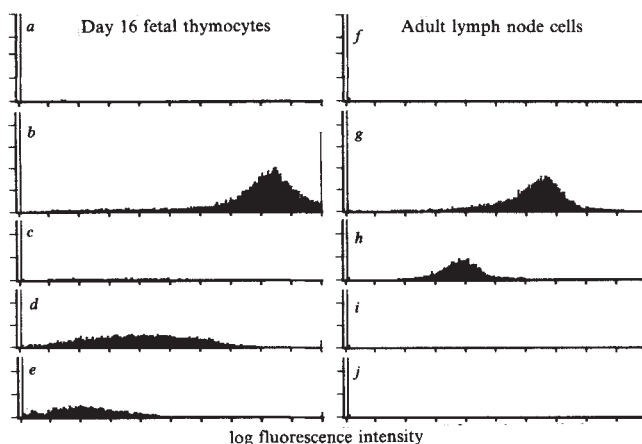


Fig. 1 Fetal thymocytes express IL-2 receptors. BALB/cAnN fetal thymocytes (a-e) from day 16 of gestation (day 0 is the day of observation of vaginal plugs) or adult (8 weeks old) lymph node cells (f-j) were stained indirectly with the following rat monoclonal antibodies: a, f, no antibody; b, g, anti-Thy-1 (T24, a rat/mouse hybridoma secreting IgG anti-Thy-1, provided by Dr Ian Trowbridge); c, h, anti-Lyt-2 (53-6.7)¹⁷; d, i, anti-IL-2 receptor (3C7)¹⁴; e, j, anti-IL-2 receptor (7D4)^{13,14}.

Methods. Cell suspensions (1×10^6 cells in 50 μ l) were incubated in phosphate-buffered saline (PBS) containing 5% fetal calf serum (FCS), 0.1% NaN₃ for 30 min at 4 °C with or without monoclonal antibodies, washed three times, and resuspended in 50 μ l of the same medium containing 25 μ g ml⁻¹ affinity-purified, mouse serum absorbed fluorescent goat anti-mouse IgG antibodies (Kirkegaard and Perry, Gaithersburg, Maryland). Following incubation for 30 min at 4 °C, the cells were washed three times, resuspended in 0.5 ml of the PBS-FCS-NaN₃ solution and analysed by flow microfluorimetry with an Ortho Cytofluorograf System 50 interfaced with an Ortho 2150 computer. The antibodies were used at the following excess concentrations as determined in pilot experiments: T24, 1/10 dilution of culture supernatant; 53-6.7, 1/5 dilution of 10-fold concentrated culture supernatant; 3C7 and 7D4, 1/50 dilution of ascites fluid.

(The smaller proportion of cells positive with 7D4 antibodies and the weaker staining intensity of these cells probably results from the inferior binding of our secondary fluorescent goat-anti-rat IgG reagent to IgM molecules; data not shown.) In contrast, expression of IL-2 receptors by adult lymph node cells is nearly undetectable (as in unstimulated splenic T cells¹⁴), reflecting the need for antigenic or mitogenic stimulation for expression of IL-2 receptors by mature T cells.

To assess expression of IL-2 receptors by immature thymocytes in the adult thymus, we purified L3T4⁻, Lyt-2⁻ thymocytes by two rounds of lysis of whole thymocyte populations with monoclonal anti-L3T4 (GK 1.5¹⁵) and anti-Lyt-2 (AD4(15)¹⁶) antibodies plus complement and stained them indirectly with monoclonal antibodies. Flow cytometric analysis of these cells, with unfractionated thymocytes for comparison (Fig. 2), indicates that cells prepared in this way are routinely ~95% Lyt-2⁻, L3T4⁻, Thy-1⁺ cells. By forward-angle scatter measurements, L3T4⁻, Lyt-2⁻ thymocytes are larger than most unfractionated thymocytes (refs 7, 12 and our unpublished data) and express Thy-1 at high levels. The background staining of these cells in the negative control results from a small number of cells (about 3-5%) binding small amounts of anti-L3T4 or anti-Lyt-2 and which presumably escaped complement lysis. As can be seen, about 30% of L3T4⁻, Lyt-2⁻ thymocytes stain with the 3C7 and 7D4 antibodies reactive with the IL-2 receptor. In contrast, very few unfractionated thymocytes stain with these reagents¹⁴ (Fig. 1). However, a few positive cells, with forward-angle scatter characteristics consistent with the size of L3T4⁻, Lyt-2⁻ thymocytes, can be seen in plots of forward-angle scatter versus fluorescence (not shown). In three experiments the proportion of positive cells in unfractionated thymocyte populations

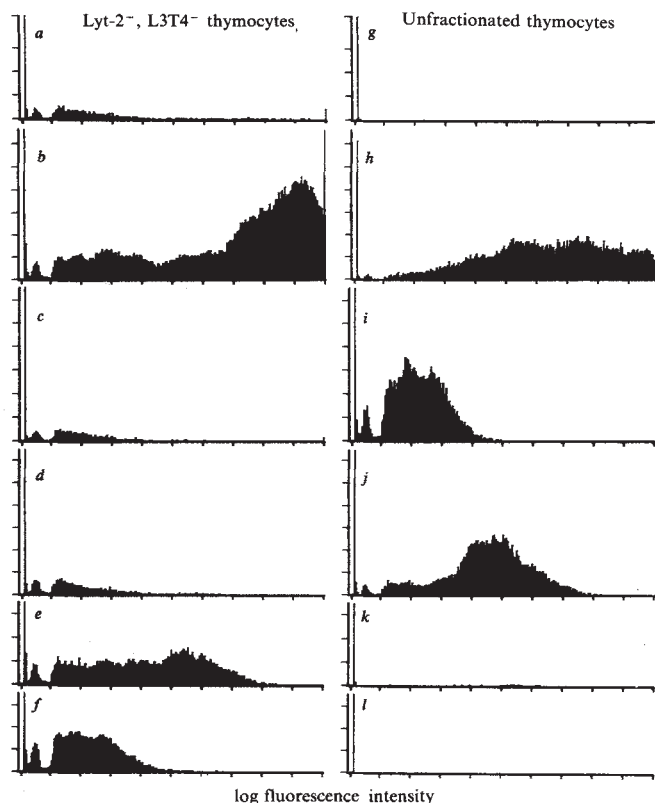


Fig. 2 Immature thymocytes in the young adult thymus express IL-2 receptors. Lyt-2⁺, L3T4⁺ thymocytes (a-f) or unfractionated thymocytes (g-l) from 4-week-old BALB/cAnN mice were stained indirectly as described in Fig. 1 legend with the following rat monoclonal antibodies: a, g, no antibody; b, h, anti-Thy-1 (T24); c, i, anti-L3T4 (1/20 dilution of 10-fold concentrated culture supernatant from the GK 1.5 hybridoma); d, j, anti-Lyt-2 (53-6.7); e, k, anti-IL-2 receptor (3C7); f, l, anti-IL-2 receptor (7D4). Concentrations of antibodies as in Fig. 1.

Methods. Lyt-2⁺, L3T4⁺ thymocytes were isolated from thymocyte suspensions by two consecutive treatments with ascites fluids (1/100 dilution) of anti-Lyt-2.2 (AD4(15))¹⁶ and anti-L3T4 (GK 1.5) plus complement (C). Cells at a concentration of $1 \times 10^7 \text{ ml}^{-1}$ in RPMI-1640 medium, 5% FCS, were incubated with antibodies at 4°C for 30 min, washed once and resuspended in a mixture of guinea pig complement (Gibco) at a 1/10 dilution and selected rabbit complement at a 1/50 dilution. Following incubation for 45 min at 37°C, the surviving cells (about 2% of the starting number) were purified on step gradients of Ficoll-Isopaque¹⁸, washed, and the entire procedure was repeated except that anti-I-A^d (1/100 dilution of ascites fluid of BP107)¹⁹ was included in the antibody cocktail in order to deplete any B cells or macrophages in the cell preparation. The overall yield of cells from the treatments was ~1% in several experiments.

averages ~1.0%. As L3T4⁺, Lyt-2⁺ thymocytes represent 3% of adult thymocytes and ~30% of these cells stain with anti-IL-2 receptor monoclonal antibody, it appears that a large fraction (or perhaps all) of IL-2 receptor-positive thymocytes are in the L3T4⁺, Lyt-2⁺ subpopulation.

As immature thymocytes are bound by two different monoclonal antibodies reactive with different epitopes on the IL-2 receptor, we predicted that these cells expressed IL-2 receptors or closely related structures. To establish whether the putative receptors bind IL-2, we exploited the observation that binding of the 3C7 monoclonal antibody to the IL-2 receptor can be blocked with purified IL-2¹⁴. Day 16 fetal thymocytes were stained indirectly with a limiting amount (as determined in a previous experiment) of 3C7 or anti-Thy-1 monoclonal antibodies in the presence or absence of 1 µg purified recombinant human IL-2. As shown in Fig. 3a-e, the binding of 3C7 monoclonal antibody but not anti-Thy-1 to fetal thymocytes is pro-

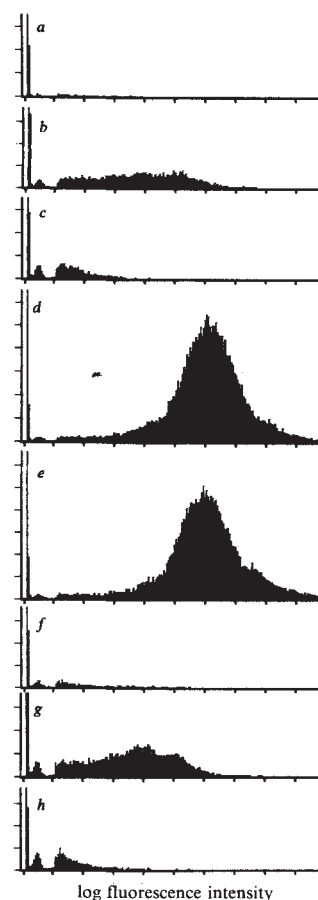


Fig. 3 IL-2 blocks binding of anti-IL-2 receptor antibodies to immature thymocytes. 1×10^6 day 16 fetal thymocytes (a-e) or young adult Lyt-2⁺, L3T4⁺ thymocytes (f-h) isolated as described in the legends to Figs 1 and 2, were preincubated for 30 min at room temperature with (c, e, h) or without (a, b, d, f, g) 1 µg purified, bacterially derived recombinant human IL-2 (AmGen Biologicals) in 50 µl of PBS-FCS- NaN_3 . Rat monoclonal antibodies were then added directly to the wells for indirect immunofluorescent staining as described in Fig. 1 legend. a, f, No antibody; b, c, g, h, a limiting (1/200) dilution of ascites fluid of the 3C7 anti-IL-2 receptor monoclonal antibody; d, e, a limiting (1/50) dilution of culture supernatant of anti-Thy-1 (T24) hybridoma.

foundly inhibited by the purified IL-2. Virtually identical results were obtained in a similar analysis of L3T4⁺, Lyt-2⁺ thymocytes isolated from young adult thymuses (Fig. 3f-h). The data, taken together, show that immature thymocytes express IL-2-binding receptors that express epitopes similar to their counterparts on activated mature peripheral T cells.

We next determined the density of IL-2 receptors on these immature thymocytes relative to concanavalin A (Con A)-activated peripheral T cells. We used the 7D4 monoclonal antibody as its binding is not inhibited by IL-2¹⁴. The antibody was used at a previously determined excess concentration. The relative level of staining of L3T4⁺, Lyt-2⁺ young adult thymocytes (a mean of 41 arbitrary fluorescence units on a linear scale) corresponded closely to the level of staining of Con A blasts (50 fluorescence units). Because in several experiments the level of IL-2 receptors on day 16 fetal thymocytes was virtually indistinguishable from the levels on L3T4⁺, Lyt-2⁺ young adult thymocytes, the fetal thymocytes also seem to express levels of receptor comparable with Con A blasts. These results indicate that IL-2 receptor expression by these immature thymocytes is at a level consistent with physiological function.

In further experiments we sought to demonstrate IL-2-dependent proliferation of adult or fetal Lyt-2⁺, L3T4⁺ thymocytes (Table 1). Given that immature thymocytes express high con-

centrations of IL-2 receptors, we were surprised to find that the cells responded only weakly to purified recombinant IL-2. Interestingly, a vigorous proliferative response of immature thymocytes to IL-2 was revealed by co-stimulation with the T-cell mitogen Con A, whereas the cells responded only weakly to Con A alone; this was true of both adult and fetal L3T4⁺, Lyt-2⁺ thymocytes. However, in two experiments the response of the fetal thymocytes to IL-2 titrated more steeply than that of the young adult L3T4⁺, Lyt-2⁺ thymocytes. Whether this reflects differences in receptor affinity or some other difference in these cell populations is unknown. We note that inclusion of irradiated T-cell-depleted syngeneic splenic accessory cells did not augment the response to Con A plus IL-2, nor did the accessory cells substitute for either Con A or IL-2 in stimulating the proliferative response (data not shown).

The expression of apparently functional IL-2 receptors by immature thymocytes suggests that IL-2 plays a role in thymus cell ontogeny. Of obvious interest in this context is the ontogeny of IL-2-producing cells in the thymus. IL-2-producing thymocytes are not detected before day 19 of fetal ontogeny and seem to correlate with the L3T4⁺, Lyt-2⁺ subpopulation⁷, but it is not clear whether the methods of that study used appropriate conditions to detect IL-2 production by immature thymocytes. We are now assessing other protocols for stimulating IL-2 production by immature thymocytes.

A possible role of IL-2 in the positive selection of thymocytes is suggested by the requirement of Con A for potent proliferative responses to IL-2 despite the fact that the cells express IL-2 receptors without *in vitro* induction. In the case of mature resting T cells, which do not constitutively express IL-2 receptors, mitogenic or antigenic stimulation leads to expression of IL-2 receptors and subsequent IL-2-dependent proliferation. Although expression of IL-2 receptors above a threshold level may be a sufficient condition for IL-2-dependent proliferation of mature T cells, our data suggest that additional requirements, provided by mitogenic stimulation, are operative, at least in the case of immature thymocytes. Con A may act by stimulating cell surface structures on immature thymocytes involved in recognition of MHC antigens and/or nominal antigens. *In vivo*,

stimulation of the receptor might aid positive selection of thymocytes during the 'education' of thymocytes.

Finally, the present results do not imply that all thymocyte proliferation is IL-2 dependent, as thymic blasts with phenotypes distinct from the L3T4⁺, Lyt-2⁺ subpopulation have been reported^{7,11}.

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Developmental regulation of T-cell receptor gene expression

David H. Raulet, Richard D. Garman, Haruo Saito & Susumu Tonegawa

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Table 1 Proliferative responses of Lyt-2⁺, L3T4⁺ thymocytes

Stimulant in culture	Lyt-2 ⁺ , L3T4 ⁺ thymocytes	
	Adult	Day 16 fetal
None	91 ± 17	52 ± 4
IL-2*, 5 U ml ⁻¹	4,518 ± 701	1,220 ± 201
IL-2, 1 U ml ⁻¹	2,477 ± 84	294 ± 61
IL-2, 0.2 U ml ⁻¹	1,131 ± 96	246 ± 187
Con A†	253 ± 26	924 ± 85.7
Con A + IL-2, 5 U ml ⁻¹	28,282 ± 1,128	18,020 ± 1,610
Con A + IL-2, 1 U ml ⁻¹	18,855 ± 1,114	1,464 ± 401
Con A + IL-2, 0.2 U ml ⁻¹	6,073 ± 634	215 ± 45

Thymocytes were treated once (fetal thymocytes) or twice consecutively (young adult thymocytes) with anti-Lyt-2 and anti-L3T4 monoclonal antibodies plus complement (see Fig. 2) and viable cells purified on gradients of Ficoll-Isopaque. Recoveries from complement lysis were 1.3% and 68% for the young adult and fetal cells respectively. In tests of stimulant activity, triplicate cultures of cells in round-bottom microtitre wells (Costar 3799 plates) contained 1×10^5 cells in 0.2 ml RPMI 1640 medium, 5% fetal calf serum, 50 μ M 2-mercaptoethanol, 0.02% glutamine, antibiotics and the additions indicated. After culture for 48 h at 37 °C in a humidified atmosphere of 5% CO₂ in air, 0.5 μ Ci ³H-thymidine (6.7 Ci mmol⁻¹) was added to each well and the cultures continued for 4 h, at which time the cells were collected and lysed on glass fibre filters and counted by liquid scintillation spectrometry.

* Purified human recombinant IL-2 (AmGen Biologicals) was titred in our laboratory and compared with a standard sample of IL-2 provided by Dr Richard Robb (Dupont, Nemours and Co.) which is referenced against the unit defined by Gillis and Smith²⁰. The specific activity of IL-2 we calculate to be 2×10^5 U per mg protein.

† Con A (Pharmacia) was added to a final concentration of 2 μ g ml⁻¹.

In contrast to B cells or their antibody products, T lymphocytes have a dual specificity, for both the eliciting foreign antigen and for polymorphic determinants on cell surface glycoproteins encoded in the major histocompatibility complex (MHC restriction)¹⁻⁴. The recent identification of T-cell receptor glycoproteins⁵⁻⁷ as well as the genes encoding T-cell receptor subunits will help to elucidate whether MHC proteins and foreign antigens are recognized by two T-cell receptors or by a single receptor. An important feature of MHC restriction is that it appears to be largely acquired by a differentiating T-cell population under the influence of MHC antigens expressed in the thymus⁸⁻¹⁰, suggesting that precursor T cells are selected on the basis of their reactivity with MHC determinants expressed in the host thymus⁹⁻¹¹. To understand this process of 'thymus education', knowledge of the developmental regulation of T-cell receptor gene expression is necessary. Here we report that whereas messenger RNAs encoding the β - and γ -subunits are relatively abundant in immature thymocytes, α mRNA levels are very low. Interestingly, whereas α mRNA levels increase during further development and β mRNA levels stay roughly constant, γ mRNA falls to very low levels in mature T cells, suggesting a role for the γ gene in T-cell differentiation.

cDNA clones encoding the α - and β -subunits of the T-cell receptor have been isolated¹²⁻¹⁷ and identified by comparison with partial protein sequences¹⁸⁻²⁰. As reported elsewhere, the predicted primary structures of the β - and α -subunits and, in the case of the β -subunit, the genomic organization of the corresponding genetic elements show striking similarities to their

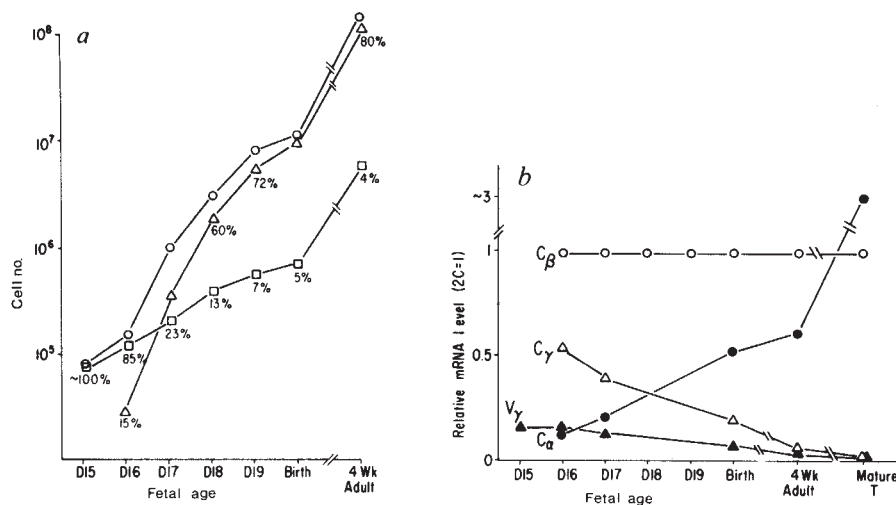


Fig. 1 *a*, Contribution of different subpopulations to thymocyte differentiation. Circles represent the approximate total cellularity of the thymus at different stages; triangles and squares represent the approximate contribution, at each stage, of 'double-positive' and 'double-negative' thymocytes, respectively. The numbers beneath the symbols represent percentages of the total. Data from Ceredig *et al.*²⁷. *b*, Approximate relative levels of α , β and γ mRNAs in thymocytes and T cells as a function of fetal and adult age. Data are from the present study. The levels are relative to RNA from the 2C line, and are determined primarily from the Northern data (Figs 3, 4) with reference to the ribonuclease assay data (Fig. 2).

immunoglobulin counterparts^{12-17,21-24}. A third gene, γ , has striking similarities to α and β , and may encode an additional T-cell receptor subunit^{15,25,26}.

Figure 1 represents our depiction of the results of Ceredig *et al.*²⁷ on the representation of different thymocyte subpopulations during murine development. Thymocytes with the Lyt-2⁻, L3T4⁻, Thy-1⁺ cell surface phenotype (hereafter referred to as 'double-negative' thymocytes) represent the large majority of thymocytes before day 17 of fetal development, and decrease in proportional representation during development until, in the young adult (4-week-old) thymus, they represent only ~4% of the total. After day 16 of fetal development, most thymocytes express the Lyt-2⁺, L3T4⁺ ('double-positive') phenotype. Thymocytes with the phenotypes characteristic of mature T cells (Lyt-2⁺, L3T4⁻ and Lyt-2⁻, L3T4⁺) are detectable shortly after this time and increase in numbers during development to represent ~15% of young adult thymocytes.

The exact lineage interrelations of these subpopulations and the identity of the direct precursor of mature peripheral T cells remain controversial issues, but recent evidence shows that double-negative thymocytes are precursors of the other thymocyte subpopulations²⁸, and presumably of peripheral T cells. In the analyses reported here, we examine RNA from thymocytes of embryos of days 15, 16, 17 and 20 (newborn) of fetal development, and of 4-week old mice. We also examine RNA from purified double-negative thymocytes from 4-week-old mice and mature lymph-node T-cell RNAs.

T-cell receptor mRNAs were detected by Northern blot analysis and with a recently developed ribonuclease protection assay. We used RNA from the cloned alloreactive cytotoxic T-lymphocyte (CTL) line 2C (ref. 29; see also refs 15, 16) as a standard in each experiment. Titrations of 2C RNA were included so that the relative amounts of each mRNA could be estimated. As a basis for comparison, the frequencies of β and γ cDNA clones in an unselected 2C cDNA library were estimated at 0.1% and 0.02% respectively (H.S., unpublished). The library has not been screened with an α probe, but the frequency of α clones in a subtracted 2C cDNA library is one-half that of β clones¹⁴.

The β probe corresponded to the last third of the β constant region and the 3'-untranslated region of the 3'-most of the two C_{β} genes (C_{β}) (Fig. 2a; refs 15, 30, 31); as these are the regions of least homology between the two genes (69%), our assays probably selectively detect transcripts of the C_{β} gene. Analysis of β -chain RNA in developing thymocytes, using the ribonuclease protection assay, showed that β -chain constant-region transcripts are already abundant at day 15 of fetal development (Fig. 2a), corresponding to about three times the level found in the reference 2C CTL line. The level of C_{β} -RNA increases somewhat on day 17 of fetal development and remains approximately constant throughout further thymocyte development. Northern blot analysis showed that approximately two-thirds of the β transcripts in RNA from day 16 fetal thymocytes are

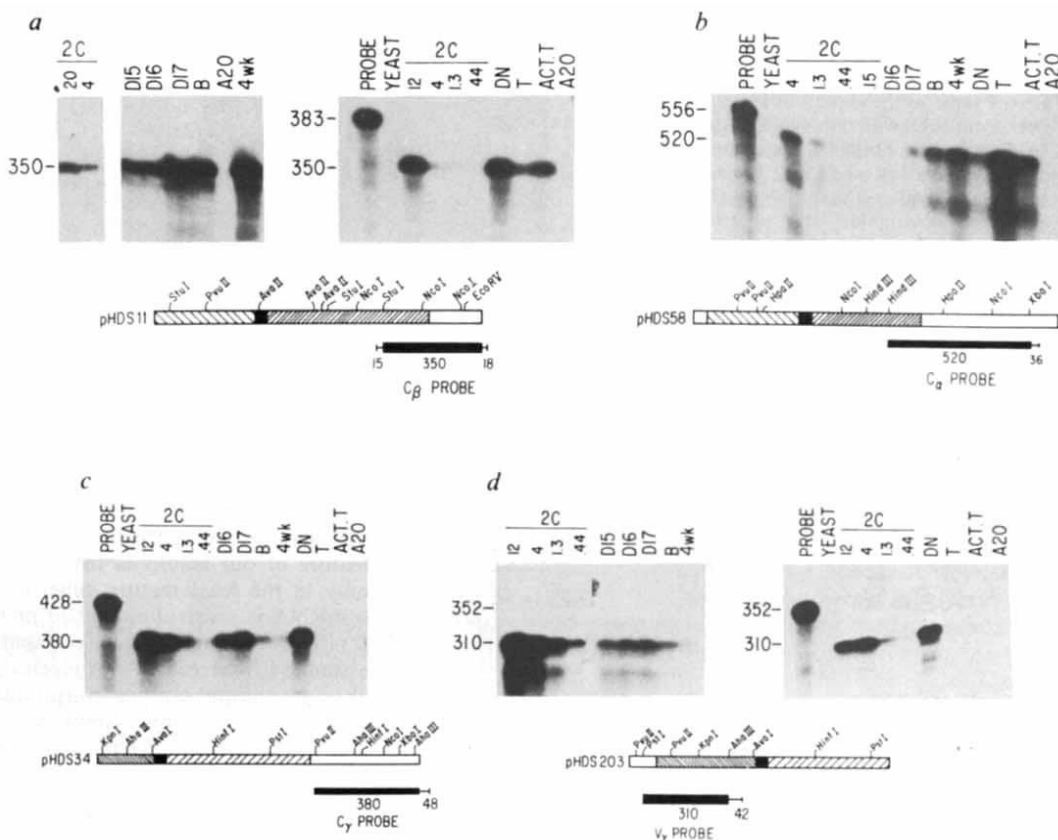
1.0 kilobases (kb) long, and about one-third of the transcripts are 1.3 kb long (Fig. 3a). Studies in the mouse²⁴ and human^{32,33} systems suggest that the functional $V-D-J-C$ transcripts of β are 1.3 kb long whereas the 1.0-kb mRNA corresponds to transcripts from incompletely rearranged loci. We estimate the level of 1.3-kb β mRNA in day 16 fetal thymocytes to be about equal to the level in RNA from the 2C cell line. The level of 1.3-kb β mRNA is roughly constant throughout further thymocyte development, and is similar in peripheral resting T cells, in T cells activated for 2 days with concanavalin A (Con A) (Figs 2a, 4b), and in the immature double-negative thymocytes from 4-week-old adult mice (Figs 2a, 4b). The fact that our β probe preferentially detects C_{β} mRNA may explain why we fail to observe higher levels of β mRNA in thymocytes than in mature T cells, as has been reported by others^{12,24}; recent studies suggest that 97% of β transcripts in murine thymocytes use C_{β} , although $C_{\beta'}$ is used in most functional T cells thus far examined³⁰.

Genomic analysis suggests that there is only a single C_{α} gene (A. Hayday, G. Tanigawa, D. Diamond, H.S. and S.T., unpublished). α mRNA is barely detectable at day 16 of development and increases in level thereafter, although even in adult thymocytes it is lower than in 2C cells (Figs 2b, 3b). The late appearance of α mRNA may explain recent data suggesting that before day 17 of fetal development, thymocytes do not express the α/β heterodimer on the cell surface³⁴. The level of α mRNA in resting or activated mature T cells is approximately three times that in 2C cells. The level of α mRNA in immature double-negative adult thymocytes is ~2-3-fold lower than that in 2C cells, or 6-9-fold lower than in mature peripheral T cells (Figs 2b, 4a).

Sequences corresponding to the constant region of the γ gene (C_{γ}) are relatively abundant in day 16 fetal thymocytes (approximately half the level in 2C by Northern analysis) and decrease in level thereafter (Figs 2c, 3c). C_{γ} mRNA is nearly 10-fold less abundant in young adult thymocytes than in 2C cells. The level of C_{γ} mRNA in adult double-negative thymocytes is the highest of any cells we have examined, corresponding to about twice the level in 2C RNA (Figs 2c, 4c). In striking contrast, the level of C_{γ} mRNA in mature T cells is extremely low (Figs 2c, 4c). A longer exposure of the gel shown in Fig. 2c was required to reveal a weak band corresponding to C_{γ} mRNA in mature T cells (not shown); we estimate the level of C_{γ} mRNA in these T cells to be more than 20-fold lower than that in 2C cells. The level of C_{γ} mRNA in T cells activated for 2 days with Con A is also very low (Figs 2c, 4c). C_{γ} mRNA levels were also very low in BALB.B lymph node T cells (not shown).

In contrast to the α and β constant-region gene segments, which appear to use a larger number of different variable-region gene segments, a single γ -chain variable-region gene segment is transcribed in each CTL clone thus far examined^{15,21}. Therefore, we have been able to analyse the regulation of V_{γ} mRNA expression in developing thymocytes. V_{γ} -containing mRNA was detected in day 15 fetal thymocyte RNA using the ribonuclease

Fig. 2 Analysis of β (a), α (b), C_γ (c) and V_γ (d) mRNA levels in BALB/c AnN thymocyte and T-cell RNAs, using the ribonuclease protection assay⁴⁰. 4 μ g of total cellular RNA, or the indicated amounts of a standard preparation of total cellular RNA from the 2C cell line, were analysed. Samples containing <4 μ g of 2C RNA were supplemented with yeast RNA. D15, D16 and D17 refer to RNAs prepared from thymocyte suspensions of fetuses killed 15, 16 or 17 days after detection of the vaginal plug (day 0). B, Newborn thymocytes (day 20 after detection of the vaginal plug); 4 wk, thymocytes from 4-week old mice; T, RNA from nylon-wool column-purified lymph-node T cells from 8-week-old mice, and ACT.T, RNA from the same cells activated for 42 h with Con A in the presence of equal numbers of irradiated splenic accessory cells; DN, RNA from 'double-negative' thymocytes purified from thymocyte suspensions from 4-week-old mice by two rounds of lysis with anti-L3T4 (GK1.5; ref. 41) and anti-Lyt-2 (AD4(15); ref. 42) monoclonal antibodies plus complement, and FicolI-Isopaque purification of viable cells. Such cells are estimated to >90% pure double-negative thymocytes by cell sorter analysis (not shown). A20, RNA from the A20 B-lymphoma cell line. A20 RNA and yeast soluble RNA (4 μ g) served as negative controls.



Methods. The probes were derived by subcloning in reverse orientation the indicated restriction fragment from pHDS 11 (β ; ref. 15), pHDS 58 (α ; ref. 17), pHDS 203 (V_γ ; ref. 15) and pHDS 34 (C_γ) downstream from the SP6 promoter in the SP6 plasmids⁴⁰. (pHDS 34 is a cDNA clone containing the 3' portion of the γ gene; ref. 25.) ³²P-labelled single-stranded (SS) RNA probes complementary to T-cell receptor mRNAs were transcribed *in vitro* from the linearized SP6 plasmids, using the SP6 polymerase, in the presence of 10 μ M ³²P-UTP (600 Ci mmol⁻¹) and excess cold ATP, GTP and CTP⁴⁰. The lengths of the probes derived from the β , α and γ cDNAs are indicated in the diagrams (blackened in), as well as the length of the SP6 plasmid sequences also included in the transcripts (flanking lines). The ³²P-labelled probes were gel-purified and hybridized for 12 h at 40 °C with total cellular RNA, isolated by the guanidinium isothiocyanate/CsCl technique⁴³ from different cell types. The hybridization mixture was then treated with ribonuclease at 22 °C to digest single-stranded RNA. The ribonuclease was destroyed by incubation with proteinase K followed by phenol-chloroform extraction, and the RNA was ethanol-precipitated. The samples were electrophoresed on denaturing 7M urea/6% polyacrylamide gels, and the gels were subjected to autoradiography. Undigested probe was run on each gel; digestion of the SP6 plasmid-derived sequences from the probe in the experimental samples confirms the specificity of the assay.

protection assay, at ~10–15% the level found in clone 2C RNA (Fig. 2d). As the level of 1.5-kb C_γ mRNA in fetal thymocyte RNA is ~50% the level in 2C cells (see above), fetal thymocytes apparently contain 1.5-kb transcripts with C_γ sequences that do not hybridize with our V_γ probe. These transcripts may represent so-called 'sterile transcripts' of the γ gene, although sterile transcripts are usually shorter than their complete counterparts^{24,32,33}. Alternatively, high-level transcription of C_γ rearranged to a distinct V_γ gene segment which does not hybridize with our V_γ probe would also account for the observed discrepancy.

V_γ mRNA declines to very low levels in adult thymocytes. Northern blot analysis confirmed that V_γ mRNA levels are low early in development compared with 2C and fall further after day 17 of fetal development (Fig. 3d). However, the level of V_γ mRNA in the adult double-negative thymocytes is much higher (approximately sevenfold) than that in fetal thymocytes (Fig. 2d). As is the case for C_γ -containing mRNAs, the level of V_γ mRNA in mature resting or activated T cells is extremely low, requiring for detection a longer exposure of the gel shown in Fig. 2d.

As the γ locus appears to undergo similar gene rearrangements on most rearranged chromosomes^{15,25}, Southern blot analysis of DNA from a T-cell population allows one to make a positive

assessment of the extent of gene rearrangement. Rearrangement of C_γ gene segments is already detectable in DNA from fetal thymocytes (day 15, as evidenced by the appearance of a faint band of high relative molecular mass, and the diminution in intensity of the 13.4-kb germline band (Fig. 4d). The frequency of rearranged alleles increases during development, but ~50% of the alleles are still unrearranged even in adult thymocytes²⁵ (Fig. 4d). Our data suggest that the level of V_γ mRNA per rearranged γ allele prior to day 17 of fetal development is roughly comparable to the level in the 2C CTL line.

Approximately 50% of the γ alleles have undergone rearrangement in double-negative adult thymocytes, paralleling the increase in V_γ mRNA in these cells compared with fetal thymocytes (which also express the double-negative phenotype). These results suggest that the double-negative subpopulation has undergone maturation between the fetal and young adult stages with respect to rearrangement and expression of the γ genes. In contrast, mature T cells have a large proportion (~70%) of rearranged γ alleles²¹ (Fig. 4d), yet have very low levels of γ mRNA.

Figure 1b shows the levels of α , β and γ mRNAs in thymocytes as a function of fetal age; the values given are rough estimates of the levels of 1.7-, 1.3- and 1.5-kb α , β and γ mRNAs respectively, relative to RNA from the 2C cell line, determined

Fig. 3 Northern blot analysis of RNA samples from developing thymocytes. The probes are: *a*, C_β ; *b*, C_α ; *c*, C_γ ; *d*, V_γ . The RNA samples are the same as those used in Fig. 2. The blots were hybridized with the same SP6-derived 32 P-labelled single-stranded probes as used in the ribonuclease assay (see Fig. 2 legend).

Methods. Total cellular RNA (5 μ g) was denatured in formaldehyde, subjected to electrophoresis in 1.1% agarose/formaldehyde gels⁴³, then transferred to nitrocellulose filters, and hybridized with 3×10^7 d.p.m. of 32 P-labelled ssRNA probes (see Fig. 2 legend and probe diagrams) essentially as described by Melton *et al.*⁴⁰, except that the hybridized filters were washed at 70 °C rather than 65 °C in $0.1 \times$ SSC, 0.1% SDS before autoradiography.

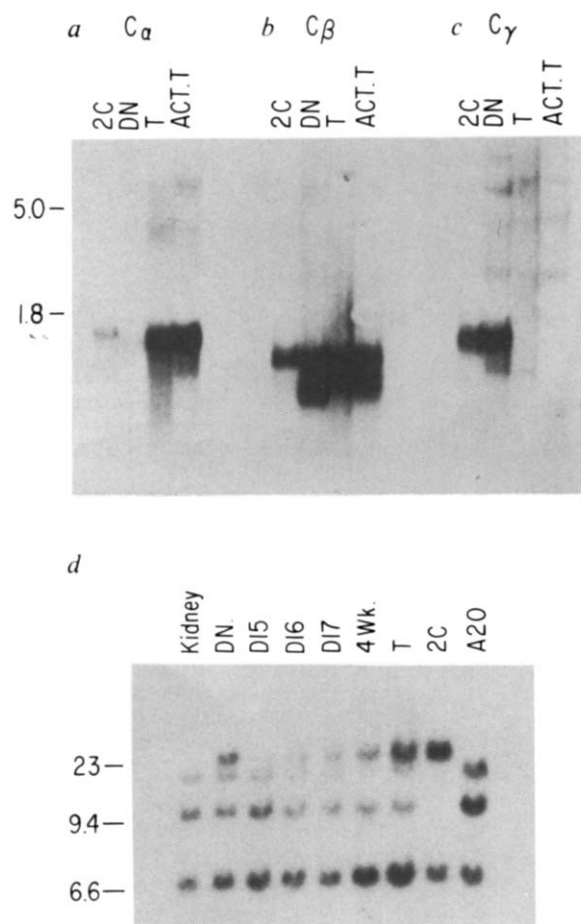
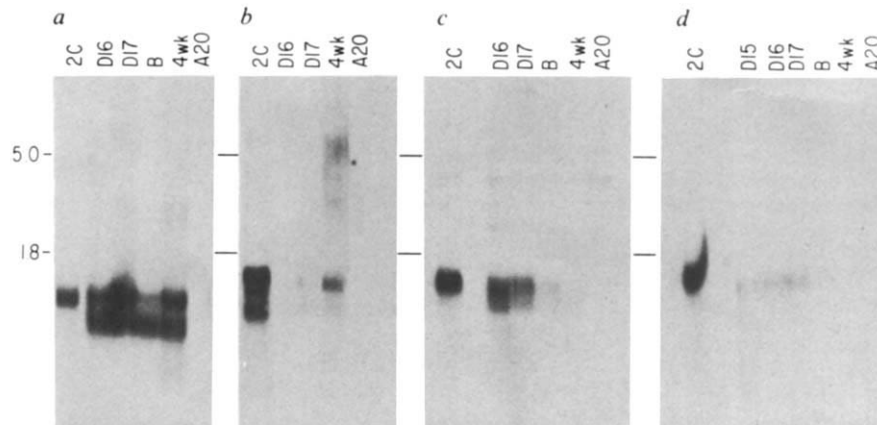


Fig. 4 *a-c*, Northern blot analysis of 5 μ g of total cellular RNA from 'double-negative' adult thymocytes (DN), lymph-node T cells (T), activated T cells (ACT.T), and 2C CTLs (2C). The probes are *a*, C_α ; *b*, C_β ; *c*, C_γ . *d*, Rearrangements of the γ gene in thymocytes and T cells. Southern blot analysis⁴⁴ of *Eco*RI-digested genomic DNA from BALB/c kidneys, fetal thymocytes (D15, D16 and D17), 4-week-old thymocytes (4 wk) and purified lymph-node T cells from 8-week-old mice (T). DNAs from the A20 B-cell lymphoma (BALB/c-derived) and the 2C line (BALB.B-derived) were also included in the analysis. BALB/c and BALB.B embryo patterns are indistinguishable²¹.

Methods. *a-c*, Northern blot analysis was performed as described in Fig. 3 legend. The preparation of the cells and RNAs were as described in Fig. 2 legend. *d*, Samples (5 μ g) of each DNA preparation were digested with *Eco*RI, electrophoresed through 0.8% agarose gels, and transferred to nitrocellulose filters. The filters were hybridized with a 32 P-labelled, nick-translated probe corresponding to the constant region of the pHDS 34 γ cDNA clone. The probe extended from the *Ava*I site near the *J* region in pHDS 34 to the 3' end (see Fig. 2c).

primarily from the Northern data (Figs 3, 4) with reference to the ribonuclease protection data (Fig. 2). The most striking feature of our results is that γ mRNA is relatively abundant only in the least mature cells of the T-cell lineage, whereas α mRNA is more abundant in mature than immature T cells. β mRNA is roughly equally abundant in each cell population examined. Whereas mRNA levels do not necessarily predict the absolute amount of the corresponding protein in a cell, the data presented here, which show shifts in the relative levels of mRNAs, probably reflect developmental regulation of the corresponding proteins.

What role might the γ gene play in T-cell recognition? Both class II-restricted helper T cells and class I-restricted CTL use a heterodimer of the α and β chains as at least part of their antigen/MHC receptor^{6,7}. Although T-cell recognition is apparently not mediated by the independent recognition of MHC and antigen by independent receptors³⁵⁻³⁷, other variations of the two-receptor model are still viable, particularly those which emphasize the constraints on physical interactions between H-2 molecules and antigens. Thus, the γ gene may encode part of a second T-cell receptor. However, based on recent evidence³⁸, it is unlikely that the specificity of either of the two putative receptors can be neatly assigned to either antigen or MHC determinants.

An alternative hypothesis is suggested by our data: perhaps the γ gene functions primarily in early T-cell differentiation. γ mRNA levels appear to be inversely related to α mRNA levels in thymocyte populations. Perhaps early in development a heterodimer of the γ and β chains is present on the surface of thymocytes prior to expression of the α gene. The putative γ/β heterodimer may serve as a receptor on immature thymocytes, allowing selection or suppression of clones expressing particular β chains. Later in thymocyte development, accompanying high-level expression of the α gene and a decrease in γ mRNA, the α -subunit may replace the γ subunit to form the receptor on mature T cells. It should be emphasized that the role of a γ -subunit in such a process need not be passive: for example, the γ chain may focus the γ/β -receptor complex onto MHC glycoproteins.

The finding that 2C and other CTL clones express relatively abundant levels of γ mRNA compared with normal T cells may result from the non-physiological growth conditions of the cells or, alternatively, induction of the γ gene in highly activated CTLs for purposes related to CTL function. However, the levels of γ mRNA are very low in T cells activated for 2 days with Con A (Figs 2, 4), which demonstrate significant CTL activity³⁹ (data not shown).

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Identification of antigen receptor-associated structures on murine T cells

James P. Allison*† & Lewis L. Lanier†

* University of Texas System Cancer Center, Science Park Research Division, PO Box 389, Smithville, Texas 78957, USA

† Becton Dickinson Monoclonal Center, Inc., 2375 Garcia Ave., Mountain View, California 94043, USA

The specific antigen receptor on human and murine T lymphocytes is a heterodimer of relative molecular mass (M_r) 80,000-90,000 (80-90K) composed of two 40-50K disulphide-linked glycoprotein subunits¹⁻⁸. Peptide map analysis of the α - and β -chains of receptor isolated from distinct tumour cell lines suggests the presence of both constant and variable regions^{2,9-12}. Unlike the antigen receptor on B lymphocytes (that is, surface immunoglobulin), the human T-cell antigen receptor seems to be non-covalently associated with another invariant structure recognized by monoclonal antibodies to the cell-surface antigens T3 and Leu 4 (refs 4, 5, 9, 12). Meuer *et al.*⁵ have demonstrated co-modulation of the T3 structure and T-cell antigen receptor using anti-clonotypic and anti-T3 monoclonal antibodies. Furthermore, immunoprecipitation with anti-T3 weakly co-precipitates a small amount of the 80-90K heterodimer in certain conditions^{4,9,12}. The murine homologue of the Leu 4/T3 structure has not been identified, although Gunter *et al.* have suggested that Thy-1 may be the counterpart of Leu 4/T3 (ref. 13). Here we describe a Leu 4/T3-like structure, distinct from Thy-1, associated with the T-cell receptor of a murine T-lymphoma cell line.

† Present address: Department of Microbiology and Immunology, University of California, Berkeley, California 94720, USA.

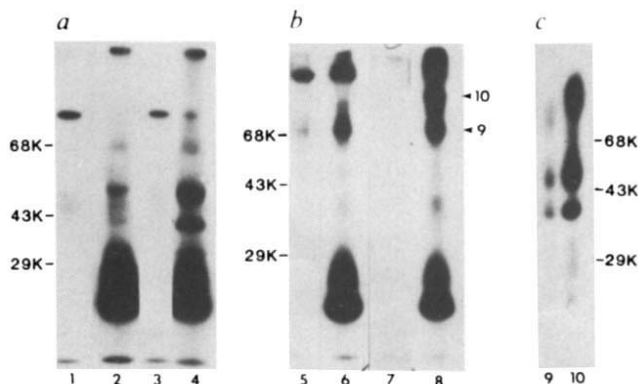


Fig. 1 SDS-PAGE analysis of human Leu 4 and associated structures. *a*, Reduced samples; *b*, non-reduced samples, run on 10% gels. Lanes 1, 2, 5, 6, sham-treated cells; lanes 3, 4, 7, 8, DTBP crosslinked cells; lanes 1, 3, 5, 7, P3 IgG1; lanes 2, 4, 6, 8, anti-Leu 4. *c*, Bands at arrows in *b* were cut out of dried gels, rehydrated in SDS sample buffer containing 50 mM dithiothreitol for 1 h, then loaded into sample wells and resolved on 12.5% gels.

Methods. Human HPB-ALL T-leukaemia cells (provided by Dr Jun Minowada) were labelled with ¹²⁵I (Amersham) using lactoperoxidase (Sigma)-catalysed radioiodination¹⁴. Half of the labelled cells were incubated for 30 min at room temperature (25°C) in phosphate-buffered saline (PBS, 0.1 M phosphate, pH 7.5) containing 10 mM DTBP (Pierce Chemicals) as described by Wang and Richards¹⁵, with minor modifications. Control cells were incubated in PBS without DTBP. After washing in 5 mM ammonium acetate/0.1 M PBS (pH 7.3) to inhibit the reaction, cells were placed in lysisate buffer (0.05 M Tris-HCl, pH 8.0) containing 0.15 M NaCl, 1 mM EDTA (Sigma), 0.02% NaN₃, 0.5% Nonidet P-40 (Sigma), 20 KIU ml⁻¹ aprotinin (Sigma) and 50 mM *N*-ethylmaleimide (Sigma) for 20 min at 4°C. Lysates were centrifuged at 13,000g for 15 min, pre-cleared for 10 min with formalin-fixed *S. aureus* (Enzyme Center), then incubated at 4°C with anti-Leu 4 antibody (Becton Dickinson Monoclonal Center) or P3X IgG1 myeloma protein. Immune complexes were isolated from lysates by incubation with rabbit anti-mouse IgG-coated formalin-fixed *S. aureus*. After washing, bound material was eluted by boiling in sample buffer containing 2.3% SDS (Bio-Rad) with or without 5% 2-mercaptoethanol (Sigma). Samples were analysed by SDS-PAGE as described by Laemmli²⁴. Protein standards (Sigma) were included for M_r determination. Gels were dried and autoradiographed at -70°C on Kodak XR film using intensifying screens (Cronex Lightning Plus).

The human T-cell antigen-specific receptor co-modulates on the cell surface with a 20-25K glycoprotein structure identified by the anti-T3 monoclonal antibody⁵. Immunoprecipitation of the T3 antigen from ¹²⁵I-labelled T-cell lines also weakly co-precipitates a small amount of the disulphide-linked 80-90K heterodimer under certain non-stringent conditions^{4,9,12}. Here, we use chemical crosslinkers to perform a nearest-neighbour analysis of structures associated with human and murine T-cell receptor. We labelled the cell surface proteins of murine or human T cells with ¹²⁵I using lactoperoxidase-catalysed radiiodination¹⁴, then crosslinked them using the cleavable reagent dimethyl dithiobispropionimidate (DTBP)¹⁵. The cells were solubilized with detergent and immunoprecipitates obtained with monoclonal antibodies and analysed by SDS-PAGE. Figure 1 shows such an analysis of human T-leukaemia HPB-ALL cells immunoprecipitated with an anti-Leu 4 antibody. Under reducing conditions (*a*), immunoprecipitates obtained from cross-linked cells contained significant amounts of two additional proteins of 37 and 45K in addition to the major Leu 4 bands at 22 and 27K. Lesser amounts of the 37 and 45K components were observed in the sham-treated controls. The properties of these proteins are consistent with the clonotypic heterodimer subunits described by Meuer *et al.*^{4,5}. Immunoprecipitations of crosslinked and sham-treated samples with monoclonal antibodies to Leu 1 (T1), Leu 2 (TB), Leu 3 (T4) and Leu 5 (T11) failed to co-precipitate the 37 and 45K components, indicating

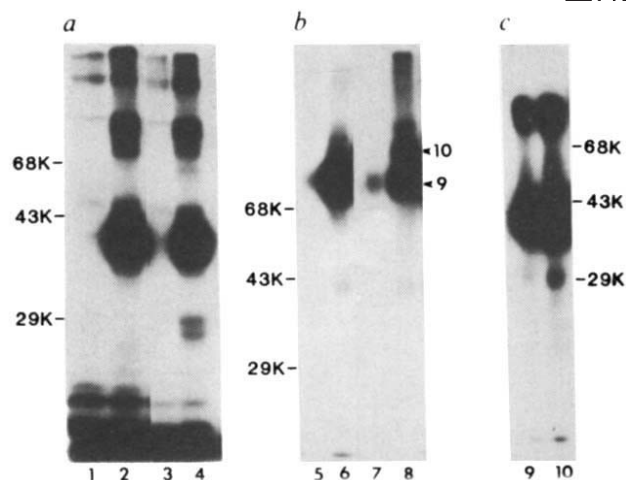


Fig. 2 SDS-PAGE analysis of T-cell receptor and associated structures from murine C6VL lymphoma cells. *a*, Reduced samples; *b*, non-reduced samples, resolved on 10% gels. Lanes 1, 2, 5, 6; sham-treated cells; lanes 3, 4, 7, 8, DTBP crosslinked cells; lanes 1, 3, 5, 7, P3 IgG1; lanes 2, 4, 6, 8, 124-40. *c*, Bands at arrows in *b* were cut out, rehydrated and reduced and contents resolved on 12.5% gels as described in Fig. 1 legend. C6VL lymphoma cells were radiolabelled, crosslinked and solubilized as described for Fig. 1. Crosslinked and sham-treated lysates were immunoprecipitated with the anti-T-cell receptor 124-40 antibody or P3X IgG1 myeloma protein.

the specificity of the reaction (data not shown). That the additional bands were obtained as a consequence of crosslinking to the Leu 4 structure is supported by additional data. As shown in Fig. 1*b*, analysis of immunoprecipitates under non-reducing conditions revealed that both crosslinked and sham-treated cells yielded, in addition to the Leu 4 bands, an additional component at 83K, as expected for the T-cell receptor heterodimer co-precipitating as a result of non-covalent association with Leu 4. The crosslinked sample, but not the sham-treated sample, yielded an additional component of 117K, a size consistent with that expected for a covalent complex of the receptor heterodimer and Leu 4. This conclusion is supported by the data in Fig. 1*c*. Cleavage of the 83K component by reduction of the gel slice followed by SDS-PAGE indicated that this component was indeed composed of the receptor heterodimer subunits, whereas similar treatment of the 117K band yielded Leu 4-like components in addition to the receptor heterodimer subunits. Taken together, these data show that chemical crosslinking can demonstrate the association between Leu 4 and receptor-like components on the surface of human T cells.

We next sought to determine whether additional structures might be associated with the murine T-cell receptor heterodimer. We have described previously the properties of a murine anti-clonotypic monoclonal antibody 124-40, which reacts exclusively with the receptor of the murine T lymphoma C6VL^{1,2}. This antibody immunoprecipitates a 80K glycoprotein composed of disulphide-linked subunits (39 and 41K)^{1,2}. C6VL cells were radioiodinated, crosslinked with DTBP, solubilized and immunoprecipitated with antibody 124-40. As shown in Fig. 2, crosslinked, but not sham-treated, samples contain two additional components of 24 and 29K that co-precipitate with the T-cell specific heterodimer. These structures are not present in the non-specific antibody control. Analysis of the samples under non-reducing conditions (Fig. 2*b*) indicated the presence of the 80K receptor heterodimer, and, in the crosslinked sample only, an additional band of ~115K consistent with that expected for the receptor heterodimer and associated structures. As shown in Fig. 2*c*, reductive cleavage of the 115K band yields a band corresponding to the receptor subunits as well as additional components similar to those obtained in Fig. 2*a*. These results

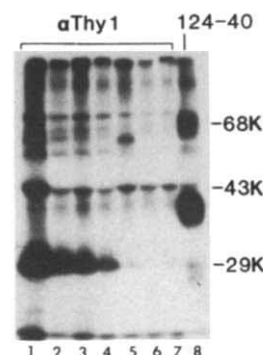


Fig. 3 Sequential immunoprecipitation with anti-Thy-1.2 and anti-T-cell receptor 124-40. Lanes 1-7, sequential immunoprecipitations with anti-Thy-1.2; lane 8, immunoprecipitation of supernatant with anti-receptor antibody 124-40. Thy-1.2 antigen was depleted from crosslinked C6VL lysate by precipitation with rat anti-mouse Thy-1.2 (clone 30-H12) antibody-coated *S. aureus*. After complete removal of all Thy-1.2 antigen, the depleted lysate was incubated with an anti-clonotypic antibody, 124-40, to the T-cell antigen receptor on C6VL, followed by rabbit anti-IgG/*S. aureus*. The bound material was eluted and analysed by SDS-PAGE under reducing conditions using a 10% polyacrylamide gel.

indicate that these structures may be a component of the murine T-cell antigen receptor complex similar to the Leu 4/T3 antigens that are associated with the human T-cell receptor heterodimer structure.

Recently, Gunter *et al.*¹³ have proposed that murine Thy-1 may be the structural and functional counterpart to the human Leu 4/T3 antigen. This hypothesis was based on the observations that: (1) Leu 4/T3 and Thy-1 are present on all mature human and murine T lymphocytes, respectively¹⁶⁻¹⁸; (2) some monoclonal antibodies against both human Leu 4/T3 and murine Thy-1 are mitogenic¹⁹⁻²¹; (3) Leu 4/T3 and Thy-1 have a similar M_r (20-30K); (4) both anti-Leu 4 and UCHL-1 antibodies react with cerebellar Purkinje cells²² (like the anti-brain reactivity of anti-Thy-1; ref. 16). However, unlike anti-Leu 4/T3 antibodies¹⁸, G7 (anti-Thy-1) described by Gunter *et al.*¹³ did not block specific T-cell antigen recognition, did not modulate readily the Thy-1 antigen on the cell surface of T lymphocytes and did not co-precipitate T-cell receptor heterodimer structures.

To address directly the relationship of the Thy-1 molecule and the two structures that co-precipitate with the T-cell receptor heterodimer from crosslinked C6VL membrane, we performed immunodepletion and peptide map analyses. As shown in Fig. 3, sequential immunoprecipitation with anti-Thy-1.2, which removed all Thy-1.2 antigen from the lysate, failed to remove the T-cell receptor heterodimer or the associated Leu 4/T3-like structures from crosslinked C6VL membrane lysates. These results indicate that the receptor is not associated with Thy-1 molecules bearing antibody-accessible 1.2 allotypic determinants. In view of the fact that the larger of the Leu 4/T3-like components was similar in electrophoretic mobility to Thy-1, it seemed possible that Thy-1 molecules, perhaps in a configuration resulting in the masking of the allotypic determinants, might comprise a portion of the receptor complex detected in the crosslinked precipitates. Therefore, we prepared peptide maps of each of the components for comparison of primary structure. The T3-like α -(29K) and β -(24K) components and Thy-1.2 were isolated from radioiodinated, crosslinked C6VL cells by immunoprecipitation with clonotypic antibody 124-40 or 30-H12, respectively, and purified by SDS-PAGE. Gel slices containing the proteins were subjected to partial proteolytic digestion and peptides resolved by SDS-PAGE as described previously²³. Maps of peptides obtained with papain, *Staphylococcus aureus* V8 protease and subtilisin are presented in Fig. 4. The patterns obtained with 24K β -T3 are clearly different from the Thy-1.2 pattern with all three proteases. For the 29K

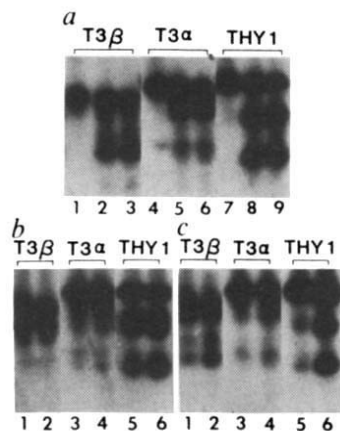


Fig. 4 Peptide map analysis of Thy-1.2 and the Leu 4/T3-like structures. *a*, Papain digests. Lanes 1, 4, 7, no protease; lanes 2, 5, 8, 5 μ g papain; lanes 3, 6, 9, 25 μ g papain. *b*, *S. aureus* V8 protease digests. Lanes 1, 3, 5, 5 μ g protease digests; lanes 2, 4, 6, 25 μ g protease. *c*, Subtilisin digests. Lanes 1, 3, 5, 10 μ g subtilisin; lanes 2, 4, 6, 50 μ g subtilisin. The Thy-1.2 and the two Leu 4/T3-structures associated with the T-cell antigen receptor on C6VL tumour were cut from gels and peptide map analysis performed essentially as described by Cleveland *et al.*²³. Peptides were resolved by SDS-PAGE on 15% gels.

α -T3, the overall patterns obtained with papain (Fig. 4*a*) and subtilisin (Fig. 4*c*) are similar to Thy-1.2, although the major fragments of Thy-1.2 are somewhat smaller. The patterns obtained with V8 protease (Fig. 4*b*), however, are quite distinct. These results indicate that the Leu 4/T3-like components are both different from Thy-1.2 in primary structure and represent distinct cell-surface components.

Our experiments demonstrate conclusively for the first time that in the mouse, as in man, additional components are associated with the heterodimer structure implicated as the T-cell antigen receptor. Further structural and functional studies of the murine equivalent of this Leu 4/T3-like structure await production of an antibody against this antigen, which will be facilitated greatly by crosslinking techniques in conjunction with anti-clonotypic monoclonal antibodies to obtain the structure for immunization.

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Direct evidence that reverse cholesterol transport is mediated by high-density lipoprotein in rabbit

N. E. Miller, A. La Ville & D. Crook

Department of Chemical Pathology and Metabolic Disorders, St Thomas' Hospital Medical School, London SE1 7EH, UK

Mammalian cells obtain cholesterol for membrane synthesis mostly via the receptor-mediated endocytosis of low-density lipoprotein (LDL)¹. Macrophages and vascular endothelium additionally have receptors that recognize certain modified forms of LDL (for example, acetyl-LDL)^{2,3}. The process by which cholesterol returns from peripheral cells to hepatocytes (reverse cholesterol transport) has not been established; although tissue culture studies have favoured high-density lipoprotein (HDL) as the principal vehicle^{4,5}, the *in vivo* evidence for this is meagre. When cholesterol-loaded macrophages are incubated in medium containing plasma, cholesterol moves from the cells to HDL and is then esterified by lecithin/cholesterol acyltransferase⁶. The accumulation of cholesteryl esters in the particles increases their size and decreases their density; enrichment with apoprotein E (apo E) also occurs, producing a decrease in electrophoretic mobility^{7,8}. We now report that similar changes occur in the circulating HDL of rabbits, when their peripheral tissues are loaded with cholesterol by intravenous (i.v.) injection of acetylated or native human LDL. This result suggests that HDL is involved in reverse cholesterol transport *in vivo*.

The experiments were carried out in six New Zealand White rabbits (*Oryctolagus cuniculus*; 3.2-4.7 kg), maintained on Sgl

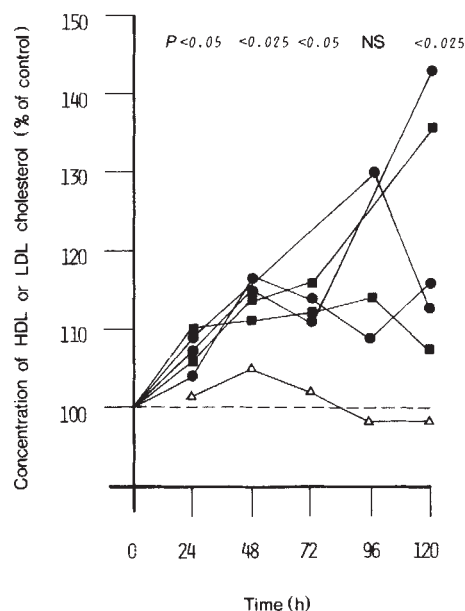


Fig. 1 Increases in the concentration of cholesterol in serum HDL measured by preparative ultracentrifugation ($d = 1.063$ - 1.21 g ml⁻¹) in five rabbits after a single i.v. injection (at time zero) of acetylated (●) or native (■) human LDL. The amount of cholesterol infused was 0.14-0.52 mmol (mean 0.29 mmol, 111 mg). The results are presented as percentages of the concentrations that existed at the time of injection. The *P* values at each time point were obtained by Student's paired *t*-test analysis of the absolute HDL cholesterol concentrations against the values at time zero. Mean concentrations of LDL cholesterol ($d = 1.019$ - 1.063), in which there was no statistically significant change, are also given (Δ). The mean HDL cholesterol concentration (with the s.e.) at 0, 24 and 120 h was 0.88 (0.22), 0.94 (0.24) and 1.06 (0.24) mmol l⁻¹ respectively; the corresponding values for LDL cholesterol were 0.79 (0.30), 0.68 (0.22) and 0.65 (0.20) mmol l⁻¹. Mean coefficient of variation of duplicate HDL cholesterol assays-3.8%.

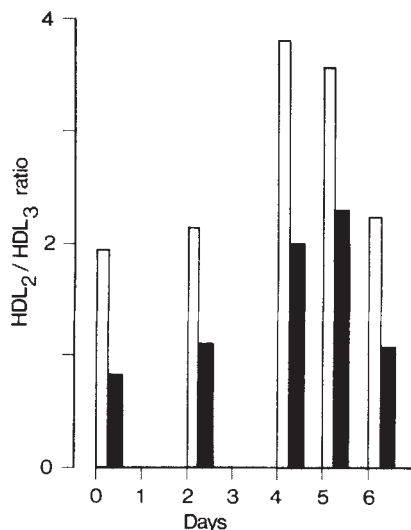


Fig. 2 Increase in the ratio of HDL₂ ($d = 1.063\text{--}1.125\text{ g ml}^{-1}$) to HDL₃ ($d = 1.125\text{--}1.21\text{ g ml}^{-1}$), measured in terms of total cholesterol content (open bars) and total protein content (closed bars) in a rabbit during the 6 days after a single i.v. injection of acetylated human LDL (0.49 mmol cholesterol, 189 mg). Results are the means of duplicate determinations.

rabbit diet (Grain Harvested Ltd, Canterbury, UK). LDL of density (d) $1.019\text{--}1.063\text{ g ml}^{-1}$ was isolated from human plasma by sequential preparative ultracentrifugation, dialysed against 0.15 M NaCl , and sterilized by filtration⁹. Acetyl-LDL was prepared using acetic anhydride, and agarose gel electrophoresis was performed to confirm that acetylation was complete¹⁰. Total cholesterol levels in serum LDL, very low-density lipoprotein (VLDL, $d < 1.006$), HDL ($d 1.063\text{--}1.21$) and the two major density subclasses of HDL (HDL₂, $d 1.063\text{--}1.125$; HDL₃, $d 1.125\text{--}1.21$) were determined by a cholesterol esterase/oxidase procedure (Boehringer-Mannheim, Cat. no. 237 574), after isolation of the lipoproteins by preparative ultracentrifugation⁹. In some animals, HDL cholesterol was also assayed after precipitation of VLDL and LDL with heparin and MnCl_2 (ref. 11). HDL protein was assayed according to Lowry *et al.*¹². Electrophoresis of serum lipoproteins in agarose gel was performed for 30 min at 90 V (15 mA) using Corning Universal Agarose Film (1% agarose, 5% sucrose and 0.035% Na_2EDTA , (w/v) in 0.065 M barbital buffer, pH 8.6), followed by staining with 0.4% Fat Red 7B in methanol. Neutral and acidic steroids in faecal collections were quantified by gas-liquid chromatography, using 5α -cholestan-3 β -ol as the internal standard^{13,14}.

Four animals were given acetylated LDL, and the remaining two native (unmodified) human LDL. All injections were made into an ear vein over 3–5 mins (total vol. 5–18 ml). The amounts infused ($0.14\text{--}0.52\text{ mmol}$ cholesterol; mean 0.32) averaged 8.4 times the circulating LDL pool. As anticipated^{15,16}, both native and acetylated human LDL were cleared rapidly from the circulation, the plasma LDL cholesterol concentrations 24 h after injection being 79–150% (mean 101%) of initial values. The rationale behind giving acetylated and heterologous LDL, rather than the native homologous lipoprotein, was to target the cholesterol load away from hepatocytes and towards other cells. Although it was not possible to examine the tissue sites of uptake in our animals, it can be assumed from previous work that most of the acetyl-LDL will have been cleared by macrophages and vascular endothelium; these are the only cell types known to possess receptors with high affinity for acetyl-LDL^{2,3,16,17}, and acetyl-LDL does not bind to the native LDL receptors of other peripheral tissues and hepatocytes^{2,17,18}. Accordingly, 10 min after injecting radioiodinated acetyl-LDL into rats, Van Berkel *et al.*¹⁶ recovered 80% of the radioactivity in the non-parenchymal cells of the liver. Using ^{14}C -cholesteryl oleate-labelled acetyl-LDL, Blomhoff *et al.*¹⁹ subsequently showed that this largely reflected uptake by vascular endothelium (60%),

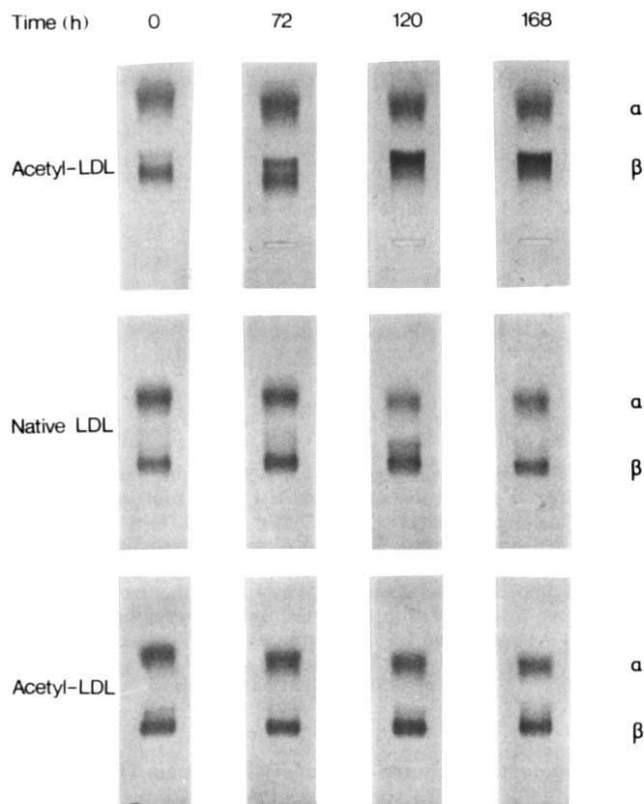


Fig. 3 Representative results from agarose gel electrophoresis of serum lipoproteins, using samples of serum collected immediately before, and at different times after, a single i.v. injection of acetylated or native human LDL into rabbits. (Serum samples were loaded at the bottom of the gels shown.) HDL migrates as the α band, and LDL as the β band. In all animals ($n = 5$) the mobility of HDL decreased progressively with time. In the pooled data, the mean electrophoretic mobilities of HDL (measured from the leading edge of the α band, and expressed relative to the pre-injection value) at 48, 72, 96, 120 and 168 h were (s.e. in parentheses): 98.5 (0.51), 96.6 (0.56), 94.0 (1.09), 91.0 (1.53) and 88.6 (1.86)%. By Student's paired t -test, the results obtained at all time points beyond 48 h were significantly different from those at time zero ($P < 0.05$ at 72, 96 and 120 h; $P < 0.02$ at 168 h). In some animals variable changes in the staining pattern of the β band were also observed; the significance of this finding, which was not associated with any change in LDL cholesterol concentration, is not clear, but one possibility is that it reflects the re-appearance in plasma of modified LDL released from vascular cells by retroendocytosis³⁴.

from which the cholesterol derived from hydrolysis of the lipoprotein was then released; uptake by hepatocytes was 15%. There also exists evidence that even unmodified human LDL is cleared largely by cells other than hepatocytes when injected into animals, although the organ sites of catabolism are distributed more widely than are those of acetyl-LDL^{15,20–22}. Consistent with these reports was our own finding that the increments in faecal cholesterol and bile acid excretion during the 72 h after the injections accounted for <6% and <12% of the administered cholesterol in the case of acetyl-LDL and native LDL, respectively.

Following clearance of the infusate from the circulation, the plasma LDL concentration showed no significant change during the following 120–144 h (see Fig. 1 legend), nor did the level of VLDL cholesterol change. In contrast, HDL cholesterol, as measured by ultracentrifugation, progressively increased in all animals by a mean of 28% (range 14–43%; $P < 0.025$) (Fig. 1); this was accompanied by a rise in the HDL₂/HDL₃ ratio, measured in terms of both cholesterol and protein content (Fig. 2), indicating a decrease in the mean hydrated density of the particles. Concomitantly, the electrophoretic mobility of HDL decreased gradually in all animals (see Fig. 3).

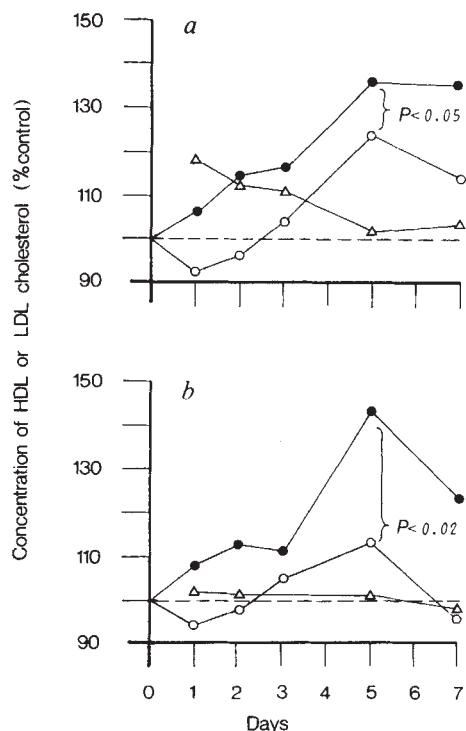


Fig. 4 Changes in the serum concentrations of HDL cholesterol (●, ○) and LDL cholesterol (△) in two rabbits, after i.v. injection of native (a) or acetylated (b) human LDL. The HDL values represented by the closed circles were obtained by ultracentrifugation ($d = 1.063\text{--}1.21\text{ g ml}^{-1}$); those represented by the open circles were obtained by assaying the cholesterol content of the supernatant after treatment of serum with heparin and MnCl_2 (ref. 11). LDL was isolated by ultracentrifugation ($d\ 1.019\text{--}1.063$). The P values were obtained by Student's paired t -test comparison of results obtained by the two different methods at the same time points. In the pooled data from four such experiments, the mean HDL cholesterol concentrations by ultracentrifugation at times 0, 72 and 120 h were $1.04\ (0.19)$, $1.18\ (0.22)$ and $1.26\ (0.17)\text{ mmol l}^{-1}$ respectively (s.e. in parentheses); the corresponding values determined using heparin- MnCl_2 were $0.98\ (0.12)$, $1.01\ (0.14)$ and $1.10\ (0.17)\text{ mmol l}^{-1}$.

These changes in HDL are identical to those that occur in animals and man during consumption of cholesterol-rich diets²³ and in tissue culture when canine plasma is incubated with cholesterol-loaded mouse peritoneal macrophages⁸. In both cases there is accumulation of large HDL particles, rich in cholesteryl esters and apo E, which *in vitro* seem to be derived from HDL₃. As these particles differ functionally from HDL₃ in being no longer able to promote a net efflux of cholesterol from cultured cells²⁴, and in being catabolized in the perfused rat liver via high-affinity apo E receptors²⁵, it has been postulated that they represent the end-products of HDL metabolism during reverse cholesterol transport.

Apo E is synthesized in several tissues at a rate which is a function of cell cholesterol content^{2,7,26}; it normally represents only a minor component of plasma total HDL, which can be divided into two subclasses on the basis of the presence or absence of the apoprotein. The results in Fig. 4 provide indirect evidence that the rise in total HDL cholesterol observed in our experiments was associated with a disproportionate increase in the apo E-containing subclass. HDL with apo E differs from HDL without apo E in being precipitable by heparin and MnCl_2 (ref. 23). When the concentrations of HDL cholesterol remaining in heparin- MnCl_2 supernatants were measured in four rabbits before and after injection of acetylated or native human LDL (two experiments with each), the relative increments were significantly smaller than those of total HDL cholesterol quantified by preparative ultracentrifugation.

Recently, the suggestion that HDL retards atherogenesis in man by removing cholesterol from the lesions²⁷ has received

increasing attention as a result of epidemiological reports of an inverse relationship between the plasma HDL cholesterol concentration and the incidence of coronary heart disease^{28,29}. Although the ability of HDL to remove cholesterol from cells is well documented *in vitro*^{4,5}, the *in vivo* evidence for such a role of HDL has been limited to demonstrations of a preferential transfer of radioactive cholesterol from tissues to plasma or lymph HDL in man^{30,31}, and to studies of the composition of peripheral lymph lipoproteins^{32,33}. Our present finding that the delivery of cholesterol to peripheral tissues is followed by changes in HDL similar to those seen when the lipoprotein is incubated with cholesterol-loaded macrophages provides the first direct evidence that the events observed *in vitro* represent the normal process of reverse cholesterol transport, and suggests a model system for studying the efficiency of the process under different conditions *in vivo*.

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Structure and transcription of human papillomavirus sequences in cervical carcinoma cells

Elisabeth Schwarz, Ulrich Karl Freese, Lutz Gissmann, Wolfgang Mayer, Birgit Roggenbuck, Armin Stremlau & Harald zur Hausen

Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, FRG

DNA of human papillomavirus (HPV) types 16 and 18 has been found closely associated with human genital cancer^{1,2}, supporting the concept that members of this virus group are key factors in the aetiology of genital cancer³. HPV 18 DNA sequences were also detected in cell lines derived from cervical cancer². We have

now analysed these cell lines, HeLa, C4-1 and 756, for the structural organization and transcription of the HPV 18 genome and we find that the HPV 18 DNA is integrated into the cellular genome and is amplified in HeLa and 756 cells. Almost the complete HPV 18 genome seems to be present in 756 cells, with the early region being disrupted into two portions in each integrated copy. In HeLa and C4-1 cells, a 2–3 kilobase (kb) segment of HPV 18-specific sequences is missing from the E2 to L2 region. HPV 18 sequences are specifically transcribed from the E6–E7–E1 region into poly(A)⁺ RNAs of 1.5–6.5 kb. Hybridization analysis of cDNA clones indicated that some of the transcripts are composed of HPV 18 and cellular sequences. In addition, poly(A)⁺ RNA hybridizing with HPV 16 DNA was found in two out of three cervical carcinoma biopsies.

Two-dimensional agarose gel electrophoresis⁴ and caesium chloride/ethidium bromide density gradient centrifugation^{5,6}, with subsequent analysis of the gradient fractions for the presence of HPV 18 sequences, showed that HPV 18 DNA is present in an integrated state within the chromosomal DNA of all three cervical cancer cell lines examined (data not shown). C4-1 cells were estimated to contain about one copy of HPV 18 DNA per cell, whereas in 756 and HeLa cells the HPV 18 sequences are amplified about 10–50-fold.

To determine which parts of the HPV 18 genome are present in the cell lines, blot hybridizations were performed using individual subgenomic fragments of HPV 18 DNA as nick-translated probes. Partial sequence analysis of prototype HPV 18 DNA and comparison with the known nucleotide sequence of the related genital papillomavirus HPV 6b (ref. 7, see Fig. 1a) allowed an unequivocal assignment of viral genome segments to each HPV 18 restriction fragment (Fig. 1b). The hybridization analysis then showed (Fig. 2) that all three cell lines contain (1) the HPV 18 non-coding region (hybridization of the cellular DNAs with the 1.1-kb *Bam*HI fragment) believed to harbour important regulatory elements for viral transcription (for review see ref. 8) and (2) the first part of the early region, including open reading frames E6, E7 and E1 (hybridization with the 2.4-kb *Bam*HI/*Eco*RI fragment). HeLa DNA seems to contain two different populations of HPV 18 sequences, one corresponding in its restriction enzyme cleavage sites (*Bam*HI, *Eco*RI) to the cloned HPV 18 prototype DNA and the other showing a modified pattern of cleavage sites (as seen by the hybridization of the 1.1-kb *Bam*HI fragment to two *Bam*HI fragments in HeLa DNA, Fig. 2B). Modifications in some HPV 18 cleavage sites were also observed in 756 (loss of the *Eco*RI site) and C4-1 DNA (data not shown). The lack of positive hybridization with the 1.4-kb *Hinc*II fragment (Fig. 2B) indicates the absence of HPV 18-specific sequences from the distal part of the early region (E2, E4) and from the late region (L2) in HeLa and C4-1 cells. In contrast, 756 cells seem to harbour most or even all of the HPV 18 DNA, as shown by hybridization of the 1.4-kb and 1.15-kb *Hinc*II fragments, respectively. The hybridization experiments suggest that HeLa and C4-1 cells contain only an incomplete HPV 18 genome with deletion of parts of the early and late region. However, we cannot rule out the possibility that these two cell lines contain the complete genome of a mosaic-like papillomavirus which, in the L1–E1 region, is composed of HPV 18-specific sequences and in the E2–L2 region is composed of sequences which do not cross-hybridize with HPV 18 in stringent conditions.

The hybridization data enabled us to deduce the possible locations of the viral integration sites. The 1.15-kb prototype HPV 18 fragment was the only probe that hybridized with two *Bam*HI fragments of 756 DNA (9 and 21 kb, respectively, Fig. 2A), indicating that opening of the circular HPV 18 DNA for integration has occurred in that region. Similarly, the hybridization patterns of HeLa and C4-1 DNA obtained with the 1.15-kb probe (Fig. 2A, B) suggest that one breakpoint of the integrated HPV 18 DNA is located downstream of the E6–E1 region, in the segment forming the 1.15-kb fragment, whereas in these two cell lines, because of the absence of some portions of HPV 18 sequences, the opposite viral integration sites are located in a

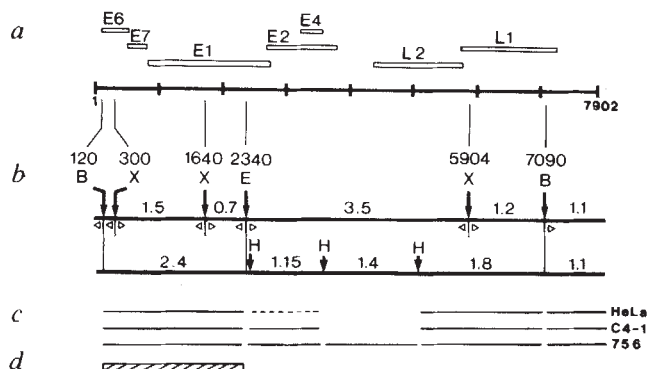


Fig. 1 **a**, Arrangement of open reading frames in HPV 6b DNA as determined by nucleotide sequence analysis⁷. E and L denote open reading frames in the putative early and late regions, respectively. The circular 7,902-base pair (bp) HPV 6b sequence is linearized at nucleotide position 1 located in the non-coding region. **b**, Alignment of HPV 18 DNA with HPV 6b according to nucleotide sequence homologies. Restriction fragments generated by cleavage with *Bam*HI (B), *Xba*I (X) and *Eco*RI (E) were used for partial sequence analysis of HPV 18 DNA (upper line). The fragment ends for which nucleotide sequence data were obtained (60–150 nucleotides from each 3'-terminally labelled end) are indicated by open triangles. 3'-Terminal labelling was performed in 50 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 1 mM dithiothreitol, with 50 μ Ci [α -³²P]dTTP (specific activity 3,000 Ci mmol⁻¹, Amersham) and 5 U DNA polymerase I (Klenow fragment, Boehringer) at 20 °C for 30 min. The partial chemical cleavage reactions for DNA sequencing were performed as described elsewhere¹⁵. The nucleotide positions in HPV 6b DNA homologous to the *Bam*HI, *Xba*I and *Eco*RI sites in HPV 18 DNA are indicated above the restriction sites. Isolated subgenomic fragments generated by cleavage of HPV 18 DNA with *Bam*HI, *Eco*RI and *Hinc*II (H, lower line) were used as nick-translated probes for hybridization with DNA and RNA from the HeLa, C4-1 and 756 cervical cancer cell lines. Sizes of restriction fragments are given in kb. **c**, **d**, Schematic summary of blot hybridization experiments performed with DNA (**c**) and poly(A)⁺ RNA (**d**) from the three cell lines. HPV 18 fragments hybridizing to cellular DNA (see Fig. 2A, B) are represented by horizontal bars. The dashed line denotes very weak hybridization. The 2.4-kb *Bam*HI/*Eco*RI fragment found to be the only fragment hybridizing to poly(A)⁺ RNA from HeLa, C4-1 and 756 cells (Fig. 3) is indicated by a cross-hatched bar.

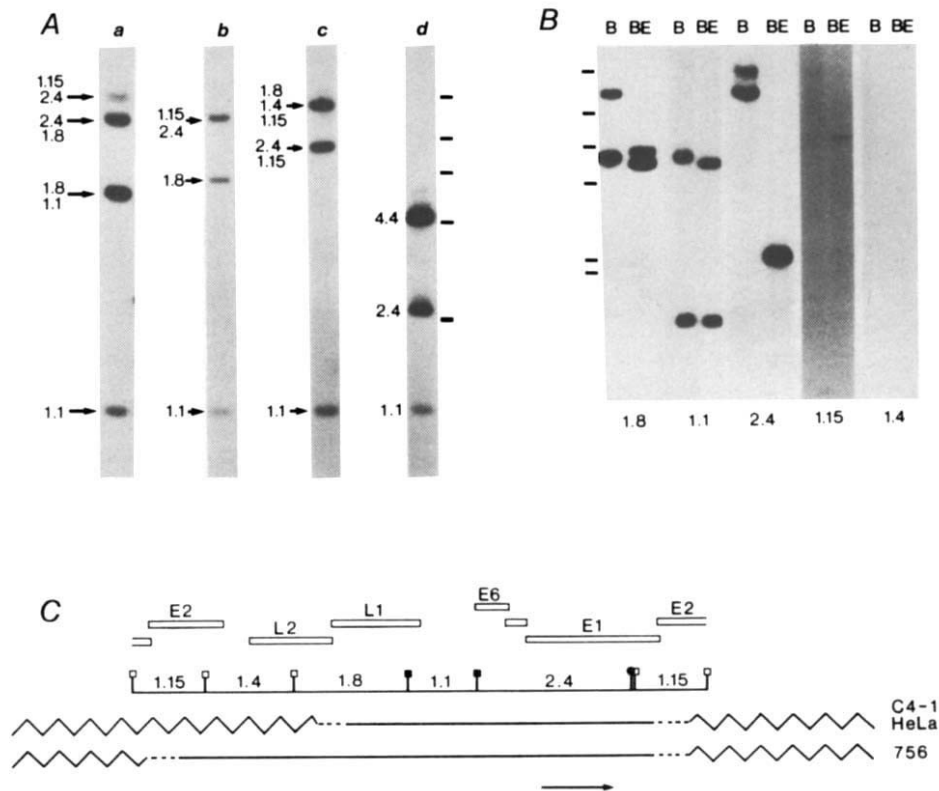
late region segment covered by the 1.8-kb *Hinc*II/*Bam*HI fragment. Alternatively, in HeLa and C4-1 cells, the transition point between the HPV 18-related and unrelated sequences of a mosaic-like papillomavirus would be located in the 1.15-kb segment.

Although the hybridization and mapping data are compatible with an overall co-linearity of the integrated HPV 18 sequences with the prototype HPV 18 DNA, they do not eliminate the possibility of sequence rearrangements that eventually may be reflected by some of the modifications in restriction enzyme cleavage sites observed in the integrated HPV 18 sequences. Figure 2C shows a scheme for the integrated HPV 18 DNA in the three cell lines. We assume that only one chromosomal integration site is present in C4-1 and 756 cells, respectively, but there is probably more than one in HeLa cells. The hybridization pattern of HeLa and 756 DNA further suggested that the amplified HPV 18 sequences are not arranged directly in tandem, but that the amplification includes flanking cellular sequences.

To analyse HPV 18 transcription in the three cell lines, poly(A)⁺ RNA was isolated and subjected to Northern blot hybridization. Hybridization with total HPV 18 DNA as probe revealed for each cell line a characteristic RNA pattern showing two or three major RNA species (HeLa, 1.6 and 3.5 kb; 756, 1.5 and 6.5 kb; C4-1, 1.5, 4.2 and 5.5 kb). When the five subgenomic fragments of prototype HPV 18 DNA (Fig. 1b) were used as nick-translated probes, exactly the same RNA pattern was obtained with the 2.4-kb *Bam*HI/*Eco*RI fragment probe

Fig. 2 **A**, Hybridization of DNA from human cervical cancer cell lines with HPV 18 DNA as probe. **B**, Hybridization of HeLa DNA with subgenomic fragments of HPV 18 DNA as probes. **C**, Possible integration patterns of HPV 18 DNA sequences into the cellular genome in HeLa, C4-1 and 756 cells as deduced from hybridization analysis with individual HPV 18 restriction fragments as probes. Cellular DNA sequences are represented by zigzag lines. Because the viral integration sites are not precisely known, the virus-cell junctions are indicated by dashed lines. A restriction endonuclease map of prototype HPV 18 DNA² is presented above, indicating the cleavage sites for *HincII* (□), *BamHI* (■) and *EcoRI* (●). Sizes of restriction fragments are given in kb. The 1.15-kb *HincII* fragment is drawn at both sites of the linearized HPV 18 DNA. The arrangement of open reading frames in HPV 6b DNA is given on top. The arrow denotes the possible direction of transcription in HeLa cells (see text). Modifications in restriction enzyme cleavage sites in the integrated HPV 18 sequences are not indicated. The existence of at least two different populations of integrated HPV 18 sequences in HeLa cells is not taken into consideration. The integrated HPV 18 sequences as shown may contain putative sequence rearrangements which would disturb the strict co-linearity with the prototype HPV 18 DNA.

Methods. **A**, Cells of the HeLa line, C4-1 line¹⁶ and 756 line¹⁷, all established from human cervical cancer biopsies, were grown in monolayer cultures in Eagle's minimum essential medium containing 10% fetal calf serum. Cellular DNA was prepared from the nuclei of cells which were used to isolate cytoplasmic RNA by incubation in 1% sarcosyl, 25 mM Tris-HCl pH 8.0, 8.3 mM EDTA pH 8.0, 100 µg ml⁻¹ proteinase K for 1 h at 37 °C. The DNA was purified by repeated extractions with phenol and chloroform: isoamyl alcohol (24:1) and was precipitated by the addition of isopropanol. Cellular DNA (10 µg) was cleaved with *BamHI*, separated on a 0.8% agarose gel and transferred to nitrocellulose filter¹⁸: HeLa (lane *a*), C4-1 (lane *b*), 756 (lane *c*), 100 pg cloned HPV 18 DNA digested with *BamHI* and *EcoRI* (lane *d*, the fragment sizes are indicated). HPV 18 DNA was purified from pBR322 vector DNA by agarose gel electrophoresis and was labelled by nick-translation¹⁹. Subgenomic fragments were prepared by digestion of HPV 18 DNA with *BamHI* and *EcoRI* and fractionation of the cleavage products in a 0.8% agarose gel. Three fragments were isolated from the gel: 2.4-kb *BamHI*/*EcoRI*, 1.1-kb *BamHI* and 4.4-kb *EcoRI*/*BamHI* fragment. The latter was further cleaved with *HincII* to prepare the 1.15-, 1.4- and 1.8-kb fragments (see Fig. 1*b*). Hybridizations were performed in 50% formamide, 5×SSC for 2 days at 42 °C. Filters were washed at 68 °C for 3×30 min in 2×SSC, 0.1% SDS and were exposed to X-ray films (Kodak X-Omat XR5) for 7 days with intensifying screens. Subgenomic fragments of HPV 18 DNA used as labelled specific probes (see Fig. 2*b* for HeLa DNA) are indicated by arrows at the respective cellular bands which they recognized. **B**, HeLa DNA 10 µg was cleaved with either *BamHI* (lanes *B*) or *BamHI* plus *EcoRI* (lanes *BE*) and separated on 0.8% agarose gels. HPV 18 restriction fragments used as nick-translated probes are indicated at the bottom. The bars indicate the λ HindIII size marker fragments (23,130, 9,419, 6,557, 4,371, 2,322 and 2,028 bp). The results of the hybridization experiments are further schematically summarized in Fig. 1*c*.



(Fig. 3, lanes *a-d*). Apart from the 1.15-kb *HincII* fragment, which gave a faint hybridization signal for the 5.5-kb RNA of C4-1, no other nick-translated fragments hybridized to any RNA. The 2.4-kb fragment contains sequences homologous to positions 120–2,340 of HPV 6b DNA, thus representing the early open reading frames E6, E7 and most of E1 (Fig. 1). The Northern analysis therefore showed that in all three cell lines HPV 18 transcription is restricted to sequences from this part of the early region (Fig. 1*d*). The HPV 18-positive RNAs were estimated to comprise 0.01–0.02% of total poly(A)⁺ RNA in 756 and C4-1 cells and ~0.2% in HeLa cells.

The orientation of the HPV 18 transcripts was analysed initially by using the 3'-terminally labelled strands of the 0.7-kb *XbaI*/*EcoRI* fragment (located within the E1 open reading frame; Fig. 1*b*) as single-stranded probes for Northern blot hybridization with HeLa poly(A)⁺ RNA. The different amounts of label incorporated into the *XbaI* and *EcoRI* fragment ends, respectively, enabled us to distinguish the two DNA strands and to predict their polarity (Fig. 4 legend). The DNA strand containing the labelled 3' *XbaI* end hybridized to the 3.5-kb RNA of HeLa, whereas the complementary strand containing the labelled 3' *EcoRI* end failed to give any hybridization signal

(Fig. 4), indicating that transcription proceeds in the sense direction through the early HPV 18 genes. The 1.6-kb RNA did not hybridize to either probe, suggesting that it does not contain sequences from the 0.7-kb segment.

The large size of some of the HPV 18-containing transcripts suggested that transcription may run further into flanking cellular (or non-HPV 18) sequences. Therefore, cDNA libraries from the poly(A)⁺ RNA of the HeLa, 756 and C4-1 cell lines were constructed and cDNA clones positive for hybridization with HPV 18 DNA isolated (E.S. and Zs. Schwarz-Sommer, to be published elsewhere). Of 18 cDNA clones from 756 cells, 7 hybridized with nick-translated total DNA from human embryonic lung cells, indicating that they contain sequences which are repeated severalfold in the human genome. Furthermore, the genomic *BamHI* fragment of 756 DNA harbouring the junction between the transcribed E6–E1 region of HPV 18 and the flanking cellular sequences also contains sequences in the cellular part which hybridize to nick-translated DNA of human embryonic lung cells (W.M., unpublished result). Together, these data strongly suggest that the seven 756 cDNA clones were derived from composite transcripts containing both early HPV 18 and cellular sequences. In contrast, all 40 HeLa and C4-1

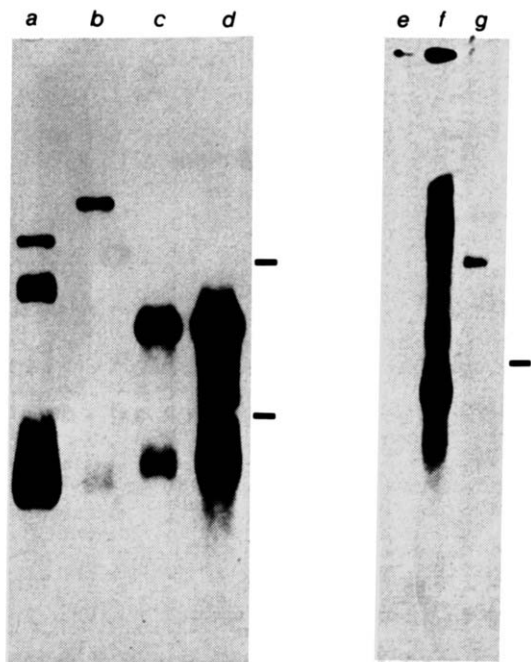


Fig. 3 Northern blot analysis of RNA from human cervical cancer cell lines (lanes a–d) and from cervical carcinoma biopsies (lanes e–g). Cytoplasmic RNA was prepared from tissue culture cells by lysis of cells in 0.6% NP40, 0.15 M NaCl, 10 mM Tris-HCl pH 7.4, 1 mM EDTA, pelleting of nuclei and repeated extractions of the supernatant with phenol-chloroform/isoamyl alcohol (24:1) as described elsewhere²⁰. Poly(A)⁺ RNA was isolated by oligo(dT)-cellulose chromatography²¹. Total RNA from tumour biopsies was prepared essentially as described elsewhere¹⁰ followed by isolation of poly(A)⁺ RNA. RNA was fractionated on 0.8% formaldehyde agarose gels²² and transferred to nitrocellulose filters, using 2 µg C4-1 (lane a), 1 µg 756 (lane b) and 0.1 µg HeLa (lane c) poly(A)⁺ RNA. In lane d, 10 µg total cytoplasmic RNA from HeLa cells was fractionated. The 2.4-kb *Bam*HI/*Eco*RI fragment of HPV 18 DNA (see Fig. 1b) was used as nick-translated probe. The bars indicate the positions of 18S (1,850 bp) and 28S (4,850 bp) ribosomal RNA used as size markers. In lanes e–g, 5 µg poly(A)⁺ RNA from each tumour biopsy were fractionated. HPV 16 DNA was used as hybridization probe which was purified from the vector DNA before nick-translation. The bar indicates the position of the 2-kb *β*-actin RNA identified by hybridization of the same filter with a nick-translated *β*-actin probe²³. Hybridizations were performed in 50% formamide, 5 × SSC for 48 h at 42 °C. Filters were washed in 2 × SSC, 0.1% SDS at 68 °C and exposed to X-ray films for 2 days (lanes a–d) or for 7 days (e–g) with intensifying screens.

cDNA clones tested were negative in the hybridization with human embryonic lung cell DNA, indicating that they lack repeated cellular sequences.

Three cervical carcinoma biopsies examined were found to harbour HPV 16 DNA (and no HPV 18 DNA), but all differed in the amount of HPV 16 DNA present and in their *Bam*HI cleavage patterns (data not shown). Northern blot analysis using total HPV 16 DNA as probe revealed poly(A)⁺ RNA species hybridizing with HPV 16 DNA in two of the samples (Fig. 3, lanes e–g). The RNAs transcribed varied greatly in amount and the major transcripts differed considerably in length. However, the small amount of DNA and RNA which could be isolated from the biopsies hampered further analysis.

The HPV 18-containing human cancer cell lines seem to differ from mouse cells transformed by bovine papillomavirus type 1 (BPV 1) and hamster tumours induced by BPV 1 in that the transcribed sequences are located in the E6–E7–E1 segment of the early region whereas the most abundant transcripts in BPV 1-transformed cells contain sequences from the end of the early region (E2, E4, E5)^{9–11}. This part of the early region, however, is uncoupled from the upstream early sequences in 756 cells by integration of the HPV 18 DNA into the cellular genome and

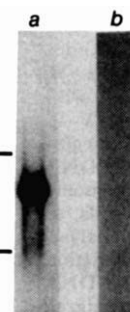


Fig. 4 Direction of HPV 18 transcription in HeLa cells. The 0.7-kb *Xba*I/*Eco*RI fragment of HPV 18 DNA (see Fig. 1b) was 3'-terminally labelled with DNA polymerase I (Klenow fragment) by incorporation of [α -³²P]dTTP. The DNA strands were separated in a 5% polyacrylamide gel in strand-separation conditions¹⁵. From the different amount of label incorporated (2 molecules TTP per *Eco*RI end and only 1 molecule per *Xba*I end), the more slowly migrating strand (1.2×10^6 c.p.m.) and the faster-migrating strand (2.3×10^6 c.p.m.) were deduced to contain the labelled 3'-*Xba*I end and 3' *Eco*RI end, respectively. Northern blots with 1 µg HeLa poly(A)⁺ RNA per slot were prepared and the filters were hybridized with the single-stranded DNA probes: lane a, slower-migrating strand labelled at the 3' *Xba*I end; lane b, faster-migrating strand labelled at the 3' *Eco*RI end. Autoradiography was for 15 days.

it is probably deleted in HeLa and C4-1 cells. Moreover, no transcripts from this region could be detected in 756 cells. Another difference is that the HPV 18 sequences are integrated into the host genome, a situation observed in most HPV 16- and HPV 18-positive cancer biopsies^{2,24}, whereas the BPV 1 genome persists mainly as an extrachromosomal episome (for review see ref. 12). In VX2 and VX7 transplantable carcinomas of domestic rabbits, however, DNA of the cottontail rabbit papillomavirus is integrated into the cellular DNA and the E6–E7 region is transcribed and spliced to the 3-terminal part of E2^{13,14}.

The transcription of specific early HPV 18 sequences in cervical cancer cell lines and the presence of HPV 16-positive poly(A)⁺ RNA species in carcinoma biopsies further point to an active role of human papillomaviruses in genital cancer.

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Human genes for U2 small nuclear RNA map to a major adenovirus 12 modification site on chromosome 17

Valerie Lindgren*, Manuel Ares Jr†, Alan M. Weiner† & Uta Francke*

Departments of *Human Genetics and †Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510, USA

U2 RNA is one of the abundant, highly conserved species of small nuclear RNA (snRNA) molecules implicated in RNA processing¹. As is typical of mammalian snRNAs, human U1 and U2 are each encoded by a multigene family. In the human genome, defective copies of the genes (pseudogenes) far outnumber the authentic genes². The majority or all of the 35 to 100 *bona fide* U1 genes³⁻⁵ have at least 20 kilobases (kb) of nearly perfect 5' and 3' flanking homology in common with each other^{4,6}; these U1 genes are clustered loosely in chromosome band 1p36 (refs 5, 7) with intergenic distances exceeding 44 kb. In contrast, the 10 to 20 U2 genes are clustered tightly in a virtually perfect tandem array which has a strict 6-kb repeating unit^{8,9}. We report here the assignment, by *in situ* hybridization, of the U2 gene cluster to chromosome 17, bands q21-q22. Surprisingly, this region is one of three major adenovirus 12 modification sites which undergo chromosome decondensation ('uncoiling') in permissive human cells infected by highly oncogenic strains of adenovirus^{10,11}. The two other major modification sites, 1p36 and 1q21, coincide with the locations of U1 genes^{5,7} and class I U1 pseudogenes¹², respectively. We suggest that snRNA genes are the major targets of viral chromosome modification.

To map the U2 genes, we hybridized a U2 probe with the chromosomes of a normal female (Fig. 1a-c). In the 119 cells analysed, 164 (31.1%) of the total 528 silver grains were located over bands 17q12-q22, with a peak at q21. This result was confirmed by hybridizing the probe with the chromosomes of a normal male: in 39 cells, 34 (22.6%) of the total 168 grains lay over bands 17q12-q22. Figure 2 illustrates the combined data for chromosome 17 from the two experiments. Although there are many U2 pseudogenes in the human genome^{2,13,14}, no secondary sites of hybridization were detected in either experiment with normal human chromosomes (data not shown). However, most U2 pseudogenes are processed genes that contain only 33-82 bp of U2 sequence and lack flanking homology to the U2

genes¹³; *in situ* hybridization probably would not detect homologies of this length.

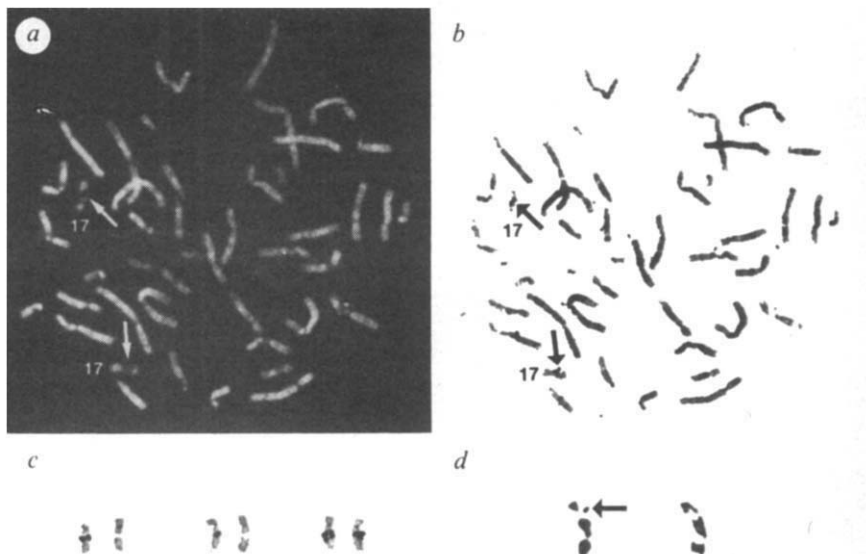
To delineate the region of assignment further, we hybridized the plasmid to cells carrying a human translocation chromosome. This chromosome, der(11), consists of the long arm of chromosome 17, from the middle of band q21 to the end of the long arm, translocated to the short arm of chromosome 11 [t(11; 17)(p15; q21)]¹⁵ (Fig. 3). Because cells from the human donor with the reciprocal translocation were no longer available, we used mouse-human hybrid cells retaining the der(11) chromosome under selective pressure¹⁶. The derivative chromosome was present at a frequency of 1.3 copies per cell. Of the 22 grains overlying this chromosome, 16 were localized over the junction region 11p15-17q22 (Figs 1d, 3). Thus, U2 genes are present in the region 17q21-q22.

The results obtained with the hybrid clone do not exclude the possibility that the U2 gene cluster was broken by the translocation in the parental human fibroblasts. The length of the U2 gene cluster (~100 kb), however, is small compared with that of a chromosome band (3,000-5,000 kb). Because the translocation breakpoint is near the middle of band 17q21, band 17q12 and the proximal portion of band q21 can reasonably be excluded as the site of the genes. In the normal lymphocytes a large proportion of the grains lay over band q21, with only a small percentage localized over q22. Therefore, the probable location of the genes is the distal half of q21.

The region 17q21-q22 containing the U2 genes is one of three major adenovirus type 12 modification sites¹¹; productive infection of human cells causes these regions to appear 'uncoiled' and often to break¹⁰. Adenovirus types 2 and 5, which are not as highly oncogenic as type 12, also induce chromosomal gaps and breaks, but apparently at random sites¹⁷. Because the thymidine kinase (TK) gene maps near the modification site on chromosome 17 and adenovirus 12 infection stimulates TK production by a factor of 2-3 (ref. 18), McDougall¹⁷ suggested that the localized alteration of chromosome structure might be related to virally induced transcription of specific host genes.

Previously, no genes had been correlated with the two other major modification sites on chromosome 1 in bands p36 and q21 (ref. 11). However, a minor modification site at 1q42 was localized immediately proximal to the 5S rRNA genes¹⁹. As shown in Fig. 4, the major modification site on the short arm of chromosome 1 coincides with the site of the U1 RNA genes in 1p36 (refs 5, 7). Further, the site in the long arm of chromosome 1, 1q21, is in the region 1q12-q22 to which we have assigned class I U1 RNA pseudogenes¹². Pseudogenes of this

Fig. 1 Human metaphase chromosomes after *in situ* hybridization with the U2 probe. **a**, Representative chromosome spread from a methotrexate-synchronized culture of phytohaemagglutinin-stimulated lymphocytes²⁴, from a normal individual, stained with quinacrine mustard dihydrochloride^{25,26}. Arrows indicate grains overlying the region 17q12-22. **b**, Cell shown in **a** after staining with 0.06% Wright stain. **c**, Pairs of chromosomes 17 from cells with grains overlying one or both homologues. **d**, der(11) chromosome, the result of the translocation t(11; 17)(p15; q21)¹⁵, with typical labelling over the arm containing bands 17q21-qter (arrow). On the right, for comparison, is a trypsin-banded example of the chromosome. The U2 probe, pBR322 derivative pML2d with an 896-base pair (bp) insert containing the entire 188-bp U2 coding sequence, hybridizes only to the U2 array in Southern genomic blotting experiments (data not shown). The plasmid was labelled by nick-translation with three tritiated nucleotides (dATP, dCTP and dTTP) to a specific activity of 2.5×10^7 c.p.m. per μg of DNA. Hybridizations were carried out for ~16 h at 37 °C at a probe concentration of 25 ng ml⁻¹ as described elsewhere²⁷. Slides were coated with emulsion and developed after 5 days of exposure at 4 °C.



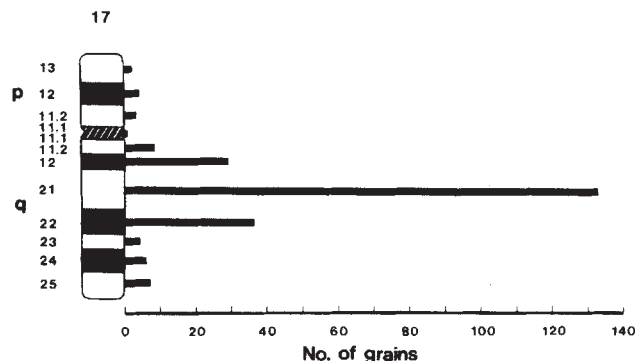


Fig. 2 Representation of chromosome 17, showing the distribution of grains after hybridization of the U2 probe with normal chromosomes from two human donors. Horizontal bars indicate the numbers of grains observed over the corresponding band in 158 metaphase spreads.

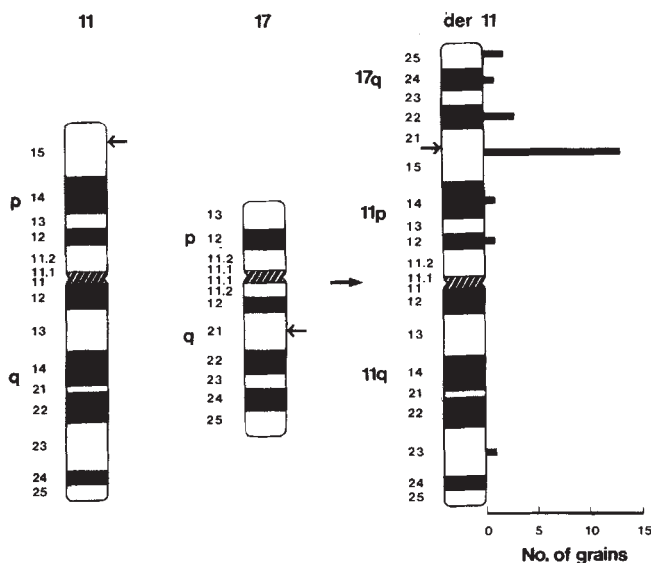


Fig. 3 *In situ* hybridization of the U2 probe with the der(11) chromosome in hybrid cells. Small arrows denote the breakpoints in bands 11p15 and 17q21 that led to the translocation t(11;17)(p15;q21)¹⁵ in the human parental fibroblasts. Hybrids between these fibroblasts and mouse thymidine kinase(TK)-deficient 3T3 cells were selected in hypoxanthine-aminopterin-thymidine medium to retain the der(11) chromosome, containing the region 17q21-qter, and/or the normal chromosome 17, both of which carry the human TK gene¹⁶. Bars next to the der(11) ideogram illustrate the numbers and positions of grains observed over 31 der(11) chromosomes in 24 hybrid cells. The peak of the grain distribution coincides with the lightly staining fusion band composed of 11p15 and 17q21.

class, unlike those of classes II and III, share extensive flanking homology with the true U1 genes^{3,6}. All four well-characterized class I U1 pseudogenes that we have mapped to 1q12-q22 cross-react with the true U1 genes at 1p36 but with no additional chromosomal sites. Thus, most or all class I U1 pseudogenes map to the third modification site. As all three of the major adenovirus 12 modification sites contain snRNA genes or very closely related pseudogenes, and one of the minor sites is near the 5S rRNA genes, we propose that these highly transcribed genes are the primary targets of the viral modification.

Studies on the structure of active chromatin lead most naturally to the hypothesis that virally induced chromosomal unfolding reflects snRNA gene activation, although gene inactivation or localized DNA replication cannot be excluded. For example, the adenovirus E1a transforming gene product may

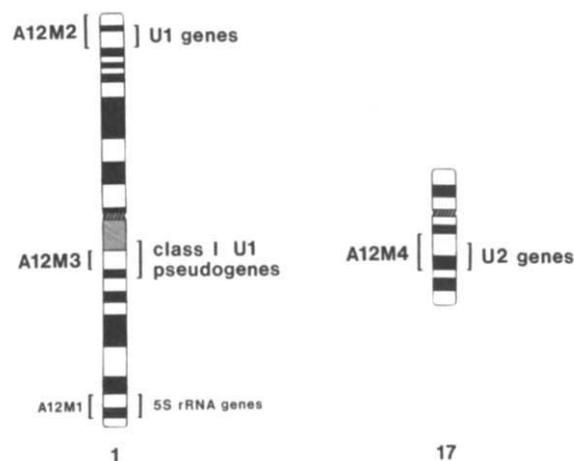


Fig. 4 Locations of human snRNA gene and pseudogene clusters correlate with the major adenovirus type 12 modification sites. As indicated by brackets, the three major viral modification sites, labelled A12M2, 3 and 4 (ref. 28), correspond exactly to the map positions of the U1 genes^{5,7}, class I U1 pseudogenes¹², and the U2 gene cluster, respectively. One of the minor modification sites, A12M1, is localized near the 5S rRNA genes¹⁹.

activate the U1 and U2 genes *in trans*, as it does a variety of other endogenous²⁰ and exogenous^{21,22} RNA polymerase II transcription units, as well as RNA polymerase III transcription units like the 5S rRNA genes (A. J. Berk, personal communication). Alternatively, the E1a product may repress snRNA expression, as it does the expression of the class I major histocompatibility antigen in infected cells²³. Class I U1 pseudogenes may be affected because they possess good flanking homology to the true U1 genes and may retain functional regulatory sequences in the DNA. An examination of the effects of viral infection on expression of host cell snRNA and of 5S rRNA may help to clarify the role of localized chromosomal uncoiling in the adenovirus life cycle.

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Market signals and subject choice

from Richard Pearson

The involvement of prospective employers in sponsoring students in higher education can lead to improvement in courses in general, as well as benefiting individuals.

In the 1980s, teachers and educationalists are expected to consider 'market signals' when planning course provision and curricula. Likewise students entering higher education are expected to adjust their subject preferences by noting changes in relative salaries, career prospects and unemployment rates. The hoped-for outcome is that educational output becomes more closely aligned to economic and labour market needs. As well as offering improved career prospects and higher salaries, employers are increasingly using sponsorship of students in higher education as a means of attracting those with scarce skills (*Nature* 307, 488; 1984). To aid this process, in 1984 the UK Department of Education and Science increased the amount a student could receive from an employer by way of a term-time bursary from £915 to £1,600 a year, with the secretary of state commenting "I am sure that these increased sponsorship levels will help to attract able students to pursue courses of great importance both to them, to employers, and to the economy..."

For individual employers, the prime motive in sponsoring undergraduates is to meet their own recruitment needs. They are now spending over £10 million each year on such activities. In 1984, one in four final-year engineering undergraduates was being sponsored, while the number of final-year electronics graduates receiving sponsorship increased by over 70 per cent in the past five years. A report shortly to be published by the Institute of Manpower Studies (see Table 1) examines the extent to which this market signal is also affecting the subject choices of those entering higher education and thus expanding the overall supply of students in poorly patronized subjects.

There was clear evidence that the number of engineering students wanting sponsorship was far greater than the number of

sponsorship places available. Given the real and perceived benefits of employer sponsorship — guaranteed industrial training, extra income, close links with an employer, and the probable offer of a job on graduation — this was not surprising (Table 1). The offer of sponsorship affected one in four students' choice of course and institution (Table 2). The biggest impact was on the choice of institution, although a significant minority switched to sandwich courses as a result of sponsorship. Of those switching subject, for a few the shift was fairly dramatic, for example from biochemistry to mechanical engineering, but for many it meant a more limited adjustment.

The impact on students depended to some extent on the way in which the employers' lists of preferred courses were drawn up. The criteria for inclusion were not only the perceived relevance or reputation of the course, but also the apparent status of the institution, the kind of student believed to be attracted to and accepted by it, the historical experience of senior managers and the recent experience of graduate recruitment personnel. Only a minority of employers included polytechnics on their preferred list.

Sponsorship can however cause difficulties for those not directly involved. Thus non-sponsored students on sandwich courses were finding it increasingly difficult to find training placements. Some teaching staff were concerned that links with a single employer might also make a student's industrial experience excessively narrow and in some cases unduly influence course content and pattern. The view was also expressed that because sponsorship focuses student and employer attention on certain, usually well known, institutions and courses, it can inhibit the development of new courses.

Because of sponsorship arrangements, each year a significant proportion of engineering graduates move directly into jobs with their sponsoring employer and effectively do not become job seekers in the annual graduate recruitment round. As a result, the number of newly qualified graduates actively seeking jobs in the labour market on graduation is considerably lower than the number graduating each year. Thus in 1983, some 500 or more newly graduating electrical/electronics engineers took jobs with their sponsoring employer while a further 600 to 800 chose to go on to research or further training or take jobs outside engineering. This meant

that employers seeking to fill direct-entry appointments were recruiting from a pool of active job-seekers totalling some 2,000 rather than 3,100 newly graduating electrical/electronics engineers.

The sponsored graduates withdrawn from the open market in this way were not evenly distributed. They tended to be among the most highly qualified young engineers in terms of course entry qualifications, and were frequently concentrated in some of the most industrially oriented and often more prestigious departments

Table 2 Influence of sponsorship on students' choices

Subject choice	14%
Type of course	25%
Institution	22%

Percentages refer to sponsored students only.

and institutions. They had also often received relevant industrial training. Non-sponsoring employers seeking to recruit from these courses, and wanting a spread of ability and educational background in their new graduate recruits, will therefore have had increased difficulties.

Overall it is clear that the availability of sponsorship has led directly to some students switching subjects and courses, a significant proportion in the case of production engineering, and acted as a positive signal to potential students about the subjects and courses employers value most. Sponsorship thus serves to increase, albeit by a small amount, the pool of students seeking to study in disciplines of the highest relevance to employers.

The content and pattern of degree courses is also being affected through the involvement of sponsoring employers, increasing their relevance to that group of employers. If these are also the most far-sighted and thoughtful employers in relation to engineering education, then the industry at large can be expected to benefit. However, sponsorship has most relevance and impact on market share, and on the quality of graduates recruited, aiding primarily the sponsoring employer and those directly involved, and has less impact on market size and the overall pool of engineering talent in the economy. □

Table 1 Students' views on the benefits of sponsorship

Extra money	55%
Practical experience	41%
Future job prospects	29%
Vacation job	24%
Better training	14%
Other	5%

Percentages are proportions of sponsored students only. These data, and data in Table 2, are from "Employers Sponsorship of Undergraduate Engineers, by A. Gordon, R. Hutt & R. Pearson (Gower, Aldershot, in the press).

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Brighton BN1 9RF, UK.